MASTER OF SCIENCE

Comparison of glycated haemoglobin and fasting blood glucose in the diagnosis of diabetes mellitus and pre-diabetes in a cohort of obese patients

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Comparison of glycated haemoglobin and fasting blood glucose in the diagnosis of diabetes mellitus and pre-diabetes in a cohort of obese patients

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SUMMARY

Diabetes mellitus is a disorder characterised by hyperglycaemia and other metabolic derangements. Diagnosis of diabetes has evolved over the years, but has been based around blood glucose measurements. Glycated haemoglobin (HbA1c) has an established role in the monitoring of glycaemia control. More recently its use as a diagnostic test for diabetes has been advocated as well. Practical advantages include the fact that fasting is not required for specimen collection. However, there are issues surrounding its use, such as standardisation and coexistent haemoglobinopathies. The aim of this study was to compare HbA1c with fasting plasma glucose in the diagnosis of type 2 diabetes and intermediate hyperglycaemia in obese patients, with a secondary aim to characterise the metabolic profile of patients identified with these criteria. This was a retrospective analysis, covering February 2010 and November 2011.

It was hypothesised that there would be some differences in the number of subjects identified in each classification (FPG, HbA1c IEC and ADA criteria), as FPG and HbA1c measure different aspects of the metabolic profile and the HbA1c IEC and ADA criteria are different in the pre-diabetes and normal categories. It was hypothesised that there would be no significant differences in the metabolic profile of the subjects identified by each classification, as subjects were from the obesity clinic and it was anticipated that the metabolic profile within

each classifications would be similar. It was also hypothesised that the number of subjects in the IFG/pre-diabetes categories fulfilling the ATP III fasting glucose criteria would be different and there would be small differences in the subjects fulfilling each available ATP criteria identified by each classification in the IFG/pre-diabetes categories, due to the reasons above.

Patients were identified from the NHS Tayside Specialist Weight Management Service. They were classified into three groups (normoglycaemia, IFG/pre-diabetes, and diabetes) according to their fasting plasma glucose (FPG) and HbA1c. The International Expert Committee (IEC) and the American Diabetes Association (ADA) have different cut-off points for diagnosis of pre-diabetes; both classification criteria were applied in the current study. Metabolic data (insulin, triglyceride, cholesterol, uric acid, alanine aminotransferase, alkaline phosphatase, sex hormone-binding globulin and testosterone) were compared and the distribution of patients in the diabetes, IFG/pre-diabetes and normoglycaemic groups were analysed.

102 subjects were classified as normoglycaemic, 13 were classified with IFG and 5 were DM by FPG; 89 subjects were classified as normoglycaemic, 21 were classified with pre-diabetes and 7 were DM by HbA1c (IEC) and 69 subjects were classified as normoglycaemic, 41 were classified with pre-diabetes and 7 were DM by HbA1c (ADA).

Significance was observed in the F-value of alkaline phosphatase in groups categorised by FPG ($p < 0.001$); significant ($p < 0.001$) F-values were found in triglyceride and alkaline phosphatase, in subjects identified by HbA1c (IEC standard) and additionally F-value in cholesterol ratio are found to be significant ($p=0.027$) in subjects identified by HbA1c (ADA standard), showing between-groups and within-group variabilities. Normality was not assessed in these subjects and ROC curves showed highly significant differences ($p < 0.001$) in triglyceride, alkaline phosphates and SHBG; significant difference ($p<0.05$) in alanine transferase in the HbA1c (IEC) categorisation. In subjects identified by HbA1c (ADA standard), the ROC curve of triglyceride level are found to be very highly significant ($p<0.001$), the ROC curve of in alkaline phosphatase and cholesterol ratio are found to be significant. The ROC curve of insulin was also shown to be significant ($p<0.05$).

Data was separated into male and female subjects, normality was assessed. The data was found to be unevenly distributed, therefore ROC curves were used to assess the data. In male subjects identified by HbA1c (IEC standard) ROC curves of triglyceride, and alkaline phosphatase were highly significant ($p<0.01$). In addition, the ROC curve of alanine aminotransferase and uric acid was significant ($p < 0.01$) and SHBG was very highly significant ($p < 0.001$). In female subjects identified by HbA1c (ADA standard), the ROC curve of triglyceride level

were found to be highly significant ($p < 0.01$); in male subjects identified by HbA1c (ADA standard), the ROC curves of triglyceride, cholesterol ratio and SHBG level were found to be significant ($p < 0.05$), the ROC curve of alanine aminotransferase was found to be highly significant ($p < 0.01$). The differences in the male groups demonstrated could be due to the small sample size in the IFG/pre-diabetes and diabetes group of subjects.

Metabolic syndrome is a complex disorder associated with insulin resistance and an increased risk of diabetes. The Adult Treatment Panel (ATP III) criteria are widely applied in the diagnosis of the metabolic syndrome. Therefore, we also examined the number of patients identified by FPG and HbA1c that fulfilled those ATP III criteria that were measured in the current study. We found that 10 out of 13 subjects (76.9%) met three out of five ATP III criteria using FPG; 8 out of 21 subjects (38.1%) using IEC, and 14 out of 41 subjects (34.2%) using the ADA criteria in the pre-diabetes group.

In conclusion, this research project compared HbA1c with fasting glucose in the diagnosis of type 2 diabetes and intermediate hyperglycaemia, in a specified cohort of patients. We identified 126 patients for inclusion into the study. Differences were observed according to the diagnostic categorisation of patients. Future recommendations include follow-up of these patients in order to observe

development of diabetes from the IFG/pre-diabetes group identified by each of the three classifications: FPG, HbA1c (IEC) and HbA1c (ADA).

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my project supervisors, **Dr Michael Murphy** and **Dr Ellie Dow** for their continued support and input throughout the project. Additional thanks are extended to **Mr George Thom** for his assistance with the database, **Mr Simon Ogston** for his help in answering my questions on statistical analyses. I would also like to thank **Dr James Walker** who inspired me on academia in diabetes and the **McInnes** family for their continuing support of my personal and academic journey. Finally, **the Royal Navy** for supporting my studies.

DECLARATION OF ORIGINALITY

This thesis constitutes original material, except where indicated in the text for illustrations and background material recreated from referenced sources. This thesis is my own work, with specific contributions as now described. The data sourced from patients attended the weight management service in NHS Tayside by medical consultants, registrars or dieticians employed by the trust. The biochemistry samples were collected, analysed and provided by NHS Tayside biochemistry department. The anthropometry data were provided by a dietician, Mr George Thom, augmented by my data gathering from the NHS Tayside medical records and also the dietetic clinic letters. Clinical information was provided by Dr Michael Murphy. I analysed and interpreted all data obtained. Statistical advice was provided by Mr Simon Ogston. Dr Michael Murphy provided supervision and advice throughout the duration of this research study.

I certify that this is a true account of my contribution to the work presented in this thesis.

Signature of Candidate……Date……

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ANOVA	Analysis of Normal Variance
ATP III	Adult Treatment Panel III
BMI	Body Mass Index
DCCT	Diabetic Control and Complications Trial
DETECT-2	Evaluation of Screening and Early Detection Strategies for Type 2 Diabetes and Impaired Glucose Tolerance
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
Hb	Haemoglobin
HbA1c	Glycated Haemoglobin
HDL	High Density Lipoprotein
IEC	International Expert Committee
NHANES	National Health and Nutrition Examination Survey

NHS	National Health Service
OGTT	Oral Glucose Tolerance Test
Q-Q Plot	Quantile-Quantile Plot
ROC	Receiver Operating Characteristic
UKPDS	UK Prospective Diabetes Study
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Background: Diabetes mellitus (DM) is a group of metabolic disorders characterised by hyperglycaemia (American Diabetes Association, 2010). Diagnosis of DM has changed over the years (World Health Organisation, 2011). Initially, the diagnosis of DM was mainly based on glycosuria (Banting FG *et al.*, 1922). Thereafter, diagnosis and treatment of DM have largely been based around measurement of blood glucose concentrations. Another measure of glycaemia is the glycated haemoglobin (HbA1c); this is a 'weighted average' of blood glucose levels for the preceding 120 days (Rohlfing *et al.*, 2000). It has been employed routinely in the monitoring of glycaemic control in diabetes management (Kilpatrick, 2004) since the late 1970s (Rohlfing *et al.*, 2002). Before the introduction of HbA1c, assessing glycaemic control relied on other tests including 24-hour urine glucose excretions (Gabbay *et al*., 1977) and daily blood glucose profiles (Gonen *et al.*, 1977), and fructosamine (Sebastian, 1999). A key advantage of HbA1c testing is that the patient does not have to fast (American Diabetes Association, 2010).

In 1997, a working party of the American Diabetes Association (ADA) suggested that HbA1c could be used for the diagnosis of diabetes (McCance *et al.*, 1997). However, in Europe, and elsewhere, it was not recommended as a diagnostic

test at the time, for a number of reasons. For example, it was difficult to set a standard cut-off point, and assays for HbA1c were not widely available in developing countries (World Health Organisation, 1999). Since then, the World Health Organisation (WHO) has held an expert consultation, which reported following a systematic review of HbA1c as a diagnostic test for DM (World Health Organisation, 2011). The consultation concluded that HbA1c can be used as a diagnostic test for diabetes, provided 'stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values' (World Health Organisation, 2011). HbA1c of 6.5% is recommended as the cut-off point for diagnosing diabetes (World Health Organisation, 2011). In January 2011 Diabetes UK endorsed the use of the HbA1c test to diagnose diabetes (John and Hillson, 2011), providing the patients are suitable and the quality control of the tests are met. Studies are required to ascertain the impact of changing from traditional means of diagnosing diabetes (fasting glucose, and/or oral glucose tolerance testing), to HbA1c.

Aims and Objectives: The aim of this project is to compare HbA1c with fasting glucose in the diagnosis of type 2 diabetes and intermediate hyperglycaemia (impaired fasting glycaemia), in our specified cohort of patients.

The objective of the project is to evaluate metabolic data collected from the NHS Tayside specialist weight management service. Metabolic measurements

including glucose, insulin, HbA1c, lipids, androgen status and renal function have been performed. The potential impact of using HbA1c versus fasting glucose will be assessed. HbA1c and body mass index (BMI) data will be compared in order to assess if the relationship between HbA1c and fasting glucose in the diagnosis of DM varies according to obesity. In addition, groups diagnosed with diabetes and IFG using each method will be compared with respect to other measures, e.g. lipids; number of patients in the intermediate glycaemia group identified by each method will be compared with the ATP III criteria.

It was hypothesised that there would be some differences in the number of subjects identified in each classification (FPG, HbA1c IEC and ADA criteria), as FPG and HbA1c measure different aspects of the metabolic profile, and the HbA1c IEC and ADA criteria are different in the pre-diabetes and normal categories. It was hypothesised that there would be no significant differences in the metabolic profile of the subjects identified by each classification, as subjects were from the obesity clinic and it was anticipated that the metabolic profile within each classifications are similar. It was also hypothesised that the number of subjects in the IFG/pre-diabetes categories fulfilling the ATP III fasting glucose criteria would be different and there would be small differences in the subjects fulfilling each available ATP criteria identified by each classification in the IFG/pre-diabetes categories, due to the reasons above.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY OF DIABETES

DM encompasses a group of metabolic disorders characterised by hyperglycemia (American Diabetes Association, 2010). It may occur as a result of defective insulin secretion, or utilisation of both insulin and fuel (insulin resistance). Diabetes, named after the Greek Aretaeus around AD100, is a very well documented disease. It was first described as 'a melting down of the flesh and limbs into the urine' (Aretaeus, N.D.). Aretaeus also identified the classical symptoms of thirst, polyuria and dehydration which are very common today (Aretaeus, N.D.). Hindu physicians, from more than 1500 years ago, described the urine as sweet-tasting (Wilding 1998), and other physicians such as Chen Chuan in the 7th century and Thomas Willis in the 17th century agreed with this (Sebastian, 1999).

A number of landmark discoveries influenced the way in which diabetes has been understood. Mering and Minkowski in 1889 discovered the urine of their pancreatectomised dogs attracted flies because it was sweet-tasting, and therefore they concluded that the pancreas is essential for the control of blood sugar concentration. In 1921 Banting and Best successfully purified insulin into an injectable form. This extract was tested on diabetic dogs, which demonstrated

a marked reduction in blood and urinary sugar levels (Banting FG *et al*., 1922). As a direct result of these early discoveries of diabetes the condition has been essentially viewed as a 'glucocentric' disorder which is primarily associated with abnormal glucose metabolism (McGarry, 1992). However, McGarry (1992) challenged this notion. He believed that abnormal fat metabolism plays an important role, at least partially, in the pathogenesis of DM.

2.2 DIAGNOSTIC CRITERIA

Hyperglycaemia is a defining feature of DM (World Health Organisation, 1999), and the advent of blood glucose measurement has meant that fasting glucose tests which demonstrate hyperglycaemia can be used to diagnose diabetes (McCance *et al*., 1997).

The diagnosis and treatment of DM have mainly been focused on glycaemic control because of the aforementioned. In the clinical setting, urinary glucose dipstick testing has been used to screen for diabetes. It is a convenient way to identify those with DM as it is a non-invasive procedure that yields instant at-the-bedside results. A positive result (glycosuria) will lead the medical practitioner to request one of the following blood tests: fasting plasma glucose, random plasma glucose test or an oral glucose tolerance test (OGTT).

2.2.1 Guidelines and Recommendations

Several guidelines regarding the diagnosis and treatment of DM have been published by the WHO since 1965 (World Health Organisation, 2006). The 1985 WHO report which defined the diagnostic criteria of DM was used for over a decade. It included symptoms such as increased thirst, and urine volume, unexpected weight loss, drowsiness, coma and high levels of glycosuria (WHO, 1985). Venous blood test results were divided into three groups: 'diabetes mellitus likely'- venous plasma glucose ≥, 11.1 mmol/l (>200 mg/dl); 'diabetes mellitus uncertain'- venous plasma glucose 5.5-<11.1 mmol/l (100-<200 mg/dl); and 'diabetes unlikely'- venous plasma glucose <5.5 mmol/l (< 100 mg/dl) (WHO, 1985). If 'diabetes mellitus is likely' a solitary result would establish the diagnosis regardless of whether symptoms are present or not (WHO, 1985). If the value lies in the 'uncertain' range, an oral glucose tolerance test (OGTT) is indicated. This involves measuring blood glucose before and 2 hours after the oral ingestion of a glucose load containing the equivalent to 75g anhydrous glucose dissolved in water (Diabetes UK, 2000). In 1997 the WHO responded to the recommendations made by the American Diabetes Association (ADA) in 1997 by suggested that the diagnosis of diabetes should not be based on a single abnormal glucose value and rather, other factors (e.g. ethnicity, family, age, adiposity and concomitant disorders) should also be considered (World Health Organisation, 1999). The WHO has since advised that the diagnostic value of the

fasting plasma glucose (FPG) should be lowered from 140mg/dl (7.8mmol/l) to 126mg/dl (7.0mmol/l), and in whole blood from 120 mg/dl (6.7 mmol/l) to 110mg/dl (6.1 mmol/l) (World Health Organisation, 1999), concurring with the ADA's recommendation. This new practice has been adopted in the UK following extensive research into complications relating to the disease (Diabetes UK, 2000).

The diagnostic criteria suggested by Diabetes UK in 2000 and the International Expert Committee IEC, in 1997 (Mayfield, 1998) are in keeping with the WHO 1999 report (World Health Organisation, 1999). These criteria include:

1. Diabetes symptoms (polyuria, polydipsia and unexplained weight loss) plus a random venous plasma glucose concentration ≥11.1 mmol/l or a FPG ≥7.0 mmol/l or two-hour plasma glucose concentration ≥11.1 mmol/l after a OGTT (Diabetes UK, 2000).

2. A definitive diagnosis should not be made in the asymptomatic patient following a single abnormal glucose test and a confirmatory plasma venous test on a separate occasion should be done (Diabetes UK, 2000). This can be in the forms of a FPG, random sample or OGTT (Diabetes UK, 2000).

Impaired glucose tolerance reflects impaired glucose regulation and is defined as a FPG <7.0 mmol/l and a glucose of 7.8-11.0 mmol/l 2 hours after the oral glucose has been administered. Impaired fasting glycaemia (IFG) has now been introduced as a comparable state of intermediate glycaemia, in order to classify individuals who have fasting glucose concentrations above the normal range but below the concentration that is diagnostic of diabetes (6.1-6.9 mmol/l).

Diabetes UK recommends that a diagnosis is confirmed by a glucose measurement performed in an accredited laboratory on a venous plasma sample although the WHO suggests whole blood is acceptable as well (Diabetes UK, 2000). There should be less need for using the OGTT (Diabetes UK, 2000), although it still remains helpful in situations where fasting glucose might not be accurate, such as in the elderly and in some ethnic minority groups (Diabetes UK, 2000).

The Scottish Intercollegiate Guidelines Network guideline (SIGN 116) on the management of diabetes follows the same principles of diagnosis as recommended by Diabetes UK. The OGTT is only used to establish diagnostic status if the random glucose value lies in an uncertain range and the fasting glucose is below the diagnostic value (Scottish Intercollegiate Guidelines Network, March 2010). SIGN 116 also recommended the changes on FPG and

whole blood as previously suggested by the WHO (1999) and Diabetes UK (2000).

2.2.2 HbA1c

HbA1c is the percentage of adult haemoglobin (Hb) that is glycated (Nathan *et al*., 2007). The glycation process involves the binding of carbohydrates non-enzymatically to proteins such as Hb (Kilpatrick, 2000). HbA1 refers to charge-separated haemoglobins of normal adult HbA_0 (Kilpatrick, 2000). Glycated Hb is a generic term for Hb bound irreversibly, in ketoamine form, to glucose (Wong WH, 1999). This includes HbA1, HbA1c and total glycated haemoglobin. HbA1 in addition may be further sub-classified as HbA1a1, HbA1a2, HbA1b and HbA1c (Kilpatrick, 2000). Total glycated Hb also includes the glycated Hb variants. HbA1c is the major sub-fraction of the glycated normal Hb (Wong, 1999). Carbohydrate, of which glucose is the major fraction, binds to Hb to form HbA1c. HbA1c was first identified as a minor fraction of normal adult Hb by ion exchange chromatography nearly 4 decades ago (Kilpatrick, 2000).

HbA1c represents the 'weighted average' of blood glucose levels over the preceding 120 days from when the test is taken (Rohlfing *et al*., 2002). It has been routinely used in the management of diabetes (Kilpatrick, 2004) since the late 1970s (Rohlfing *et al*., 2002). Previously, monitoring long-term glycaemic

control relied on tests such as ‘24 hour urinary collections’ and ‘daily blood glucose profiles’ (Kilpatrick, 2004) and fructosamine (Kilpatrick *et al.*, 1996). In comparison, HbA1c is a more reliable measure of glucose control, no fasting is required and only requires a single venous blood sample (ADA, 2010). HbA1c is a well-established and documented test for monitoring diabetes but its importance and potential use as a primary diagnostic tool is yet to be determined.

The big change in 1997 was the recommendations made by the ADA:

(a) Change ‘fasting glucose’ criterion from 7.8 mmol/L to 7.0 mmol/L.

(b) Abandon the OGTT and diagnose diabetes by FPG alone.

Some experts believed that HbA1c could be used for the diagnosis of diabetes but there were concerns due to limitations of the test (Mayfield, 1998): HbA1c is a dynamic test affected by age, race, pregnancy, different laboratory standards and co-morbid disease such as haemoglobinopathies and renal failure (Nielsen *et al*., 2004). Haemoglobinopathies, which are particularly prevalent in Africa, the Mediterranean and South East Asia, iron deficiency and haemolytic anaemia (Kilpatrick *et al*., 2009, Coban *et al*., 2004) can affect HbA1c levels. Renal failure can affect HbA1c levels via haemolysis and iron deficiency and also through the formation of carbamylated haemoglobin (Kilpatrick *et al*., 2009). HIV patients who are taking anti-viral drugs can have 1% lower HbA1c (Kilpatrick *et al*., 2009). The IEC have suggested that ageing affects HbA1c with a 0.4% increase in 70 year

olds compared to 40 year olds with the same glucose tolerance. In addition, a 0.4% HbA1c increase is also seen in Afro-Caribbeans comparing to Europids (Kilpatrick *et al*., 2009). HbA1c levels are significantly decreased in pregnancy, compared to age-matched non-pregnant women. This is reflected by a study that identified varying ranges of HbA1c in non-pregnant women (4.7-6.3%), in early pregnancy (4.5-5.7%) and in late pregnancy (4.4-5.6%) (Nielsen *et al*., 2004, Kilpatrick *et al*., 1998).

Although a HbA1c value of 6.5% (48 mmol/mol) is recommended as the cut-off point for diagnosing diabetes, a value of less than 6.5% does not exclude diabetes (World Health Organisation, 2011b). The WHO also concluded that there was insufficient evidence to make any formal recommendations on the interpretation of HbA1c levels below 6.5% (World Health Organisation, 2011b). Further work by the WHO concluded that HbA1c can be used as a diagnostic test, provided 'stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values' (World Health Organisation, 2011).

2.2.3 Pros and Cons of Diagnostic Tests for DM

Glucose as a diagnostic tool directly measures the glucose molecules which are thought to be the direct cause of many of the complications of DM. Complications

result from the formation of reactive free radicals generated by hyperglycaemia (Brownlee, 2005). Epidemiological studies of diabetic retinopathy have identified a threshold glucose level at which retinopathy becomes apparent (American Diabetes Association, 1997). A direct relationship exists between the degree of plasma glucose control and the risk of retinal, renal and neurologic complications (UK Prospective Diabetes Study (UKPDS) Group, 1998).

Some studies have shown that higher glucose concentrations are linked to a poorer prognostic outcome (Sasaki, 1981). Therefore the glucose measurement is a useful tool to predict the consequences of DM. Glucose measurement is not affected by non-glycaemic factors (e.g. haemoglobinopathies) and it demonstrates fewer inter-laboratory differences in the UK than HbA1c. In addition glucose measurements are more comparable from laboratories around the world in comparison to HbA1c results (Kilpatrick *et al*., 2009). However, glucose testing as a solitary measurement requires the patient to fast for at least 8 hours prior to sampling, with no calorie intake (American Diabetes Association, 1997). This can be a challenge for some patients.

Diurnal variation of FPG may manifest as a higher FPG in the morning and a lower FPG in the afternoon. This means the testing must be standardised for quality control of results and all patients should be seen at the same time on successive occasions (Troisi *et al*., 2000). Plasma glucose samples need to be

analysed promptly as glycolysis can cause the concentration in ex-vivo blood to decrease. Fluoride can help maintain long-term glucose stability and is usually added to sampling bottles to preserve it. However, the greatest decline in glucose concentration occurs in the first hour from venepuncture (Chan *et al*., 1989).

Oral glucose tolerance testing (OGTT) is required when assessing an individual for impaired glucose tolerance. The OGTT formally assesses whether individuals have impaired glucose tolerance. However, it is inconvenient, has poor reproducibility (Ganda *et al*., 1978), is affected by variations in plasma glucose concentrations and by ambient temperatures (Moses *et al*., 1997). Glucose measurements represent the blood sugar concentrations at two points in time (0 minutes and 120 minutes). But individual variability may render these results less reliable than HbA1c and oral glucose tolerance test such as those who have undergone gastric surgery (Kilpatrick *et al*., 2009).

HbA1c is a well-established DM monitoring tool for DM (Mayfield, 1998). HbA1c has been found useful in the identification of patients at risk of retinopathy and nephropathy (McCance *et al*., 1994). In 2009, the ADA started using HbA1c as a diagnostic tool for DM. Recently, the IEC extensively reviewed all the epidemiological evidence associated with HbA1c and found that it provides a reliable measurement of chronic glycaemia and suggested it may be a better

means of diagnosing DM (Fonseca *et al*., 2009). Diabetes complications were examined: HbA1c is just as predictive of retinopathy as glucose measurement (The International Expert Committee, 2009). As discussed, HbA1c represents the percentage of adult haemoglobin which is glycated (Nathan *et al*., 2007) and haemorrhage and blood transfusion can confound results (The International Expert Committee, 2009).

HbA1c has its strengths. As mentioned, unlike the OGTT and FPG, no prior preparation or fasting is required. (American Diabetes Association, 2010). While collecting a blood sample for HbA1c, other biochemistry tests can be concurrently collected. HbA1c is collected and stored in tubes with ethylenediaminetetraacetic acid (EDTA), which acts as an anticoagulant. In summary, the HbA1c test is a clinically convenient test with less pre-analytical instability (The International Expert Committee, 2009).

Other tests are available which may be useful in specific circumstances. ***Fructosamine*** reflects glycaemic control over the preceding 1-3 weeks (Kilpatrick *et al*., 1996) and it is used in pregnancy and in those suffering with haemoglobinopathies to assess control over a shorter period than HbA1c (Longsmore M, 2008). Older studies in the past had also used ***Mean Amplitude***

of Glycaemic Excursions (MAGE) as an objective system to quantify glycaemic instability (Service *et al*., 1970).

The limitations and uncertainties linked to the use of HbA1C as a single diagnostic test have been enough to defer its adoption in the United Kingdom (UK) to diagnose diabetes. In January 2011 Diabetes UK endorsed the use of the HbA1c test to diagnose diabetes (John and Hillson, 2011) with several qualifications/assumptions: that the patients are suitable, that staff are trained and that the method can demonstrate 'internal quality control and external quality assessment performance that matches a laboratory method' (World Health Organisation, 2011b). Most, but not all, patients are suitable for the test; however, as HbA1c reflects the chronic glycaemic state it is not suitable in situations where blood sugar concentrations have risen rapidly (John and Hillson, 2011). For example symptomatic children and young people, those with a short duration of symptoms, acutely ill patients with a high risk of diabetes, pancreatic pathologies and those taking medications that may stimulate a rapid rise in glucose (e.g. corticosteroids) (John and Hillson, 2011) are all deemed unsuitable.

HbA1c is a robust tool that reflects chronic glycaemia. There are strong correlations between HbA1c and retinopathy (The International Expert Committee, 2009), and it is at least as predictive of retinopathy as glucose-based tests (Kilpatrick *et al*., 2009). There is a lower within-person variability than

glucose. In the past, it was considered that there was lack of standardisation and normal ranges, therefore it was not used as a diagnostic tool (Mayfield, 1998). However, with a new reference method and standardisation led by IFCC (Kilpatrick *et al*., 2009) to calibrate HbA1c instruments, these issues are being addressed (The International Expert Committee, 2009).

2.3 OBESITY AND DIABETES

Associations has been made between obesity and diabetes for more than 1500 years by Ayurvedic (Hindu) physicians, and it has been described as 'a syndrome affecting older overweight patients who passed large volumes of sweet-tasting urine' (Wilding J, 1998). In modern days, obesity plays a major role in 80% of type 2 diabetes, which remains the most important problem in diabetes management world-wide (Wilding J, 1998).

Studies have confirmed that the risk of developing type 2 diabetes rises progressively with increased BMI and waist circumference (Chan *et al.*, 1994). In a prospective cohort study, it has been demonstrated that weight gain in adulthood increases the risk of DM significantly, while weight loss can reduce the risk of DM (Colditz *et al*., 1995). The distribution of adipose tissue plays a role in the susceptibility to type 2 diabetes (Vague, 1956). Central obesity (android

obesity or ‘apple-shaped’) (Vague, 1956) is recognised as carrying an increased risk of metabolic syndrome, glucose intolerance and diabetes (Reaven, 1988, Després *et al.*, 2008).

CHAPTER 3

SUBJECTS AND METHODS

This chapter details the subjects and methods used in this study. It outlines the selection of subjects and the methods used to compare the use of glycated haemoglobin (HbA1c) and fasting glucose measurements in the diagnosis of type 2 diabetes and intermediate categories of hyperglycaemia (e.g. impaired fasting glycaemia).

3.1 SUBJECTS

The dataset for this study was assembled in the following way. First, patients were identified from the Tayside Specialist Weight Management Service database, having attended between February 2010 and November 2011 as new referrals. Subjects were included if either of the following criteria were met:

(i) Body mass index (BMI) ≥40 kg/m^2, irrespective of co-morbidity, or

(ii) ≥35 kg/m^2, with at least one relevant co-morbidity (e.g. cardiovascular disease, hypertension, hypercholesterolaemia). Their biochemical results were stored in the biochemistry database by the NHS Tayside Department of Biochemical Medicine. These results included fasting glucose, insulin, lipids, liver function tests, uric acid and androgen status.

In those cases where the BMI information had not been recorded on the request form, this information was extracted from other sources (clinic letters from dietetic and medical records). Clinical information i.e. whether the patients already have been diagnosed with diabetes, was established using information from biochemistry and dietetic sources.

Approval by the University of Dundee Caldicott Guardian was sought and gained before data collection commenced.

The subjects were given study numbers thereby ensuring the anonymity of the patients involved in this study. The dataset was stored on a secure hard drive to which only the researcher had access.

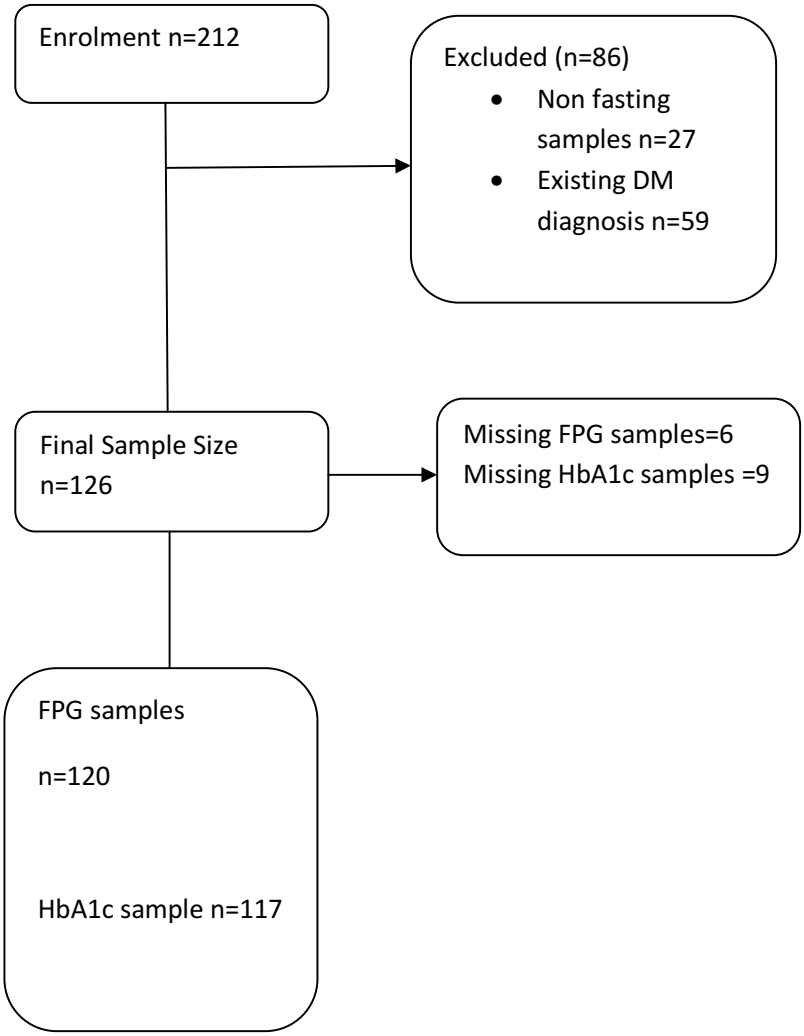

Figure 1: Console flow diagram

3.2 METHODS

The initial stage of the project involved data collection. A spreadsheet was constructed. Patients were identified from their first visit to the weight management service and the biochemical (metabolic) results of fasting venous plasma blood samples were accessed. These baseline metabolic profiles are routinely performed on every patient attending the weight management service for the first time.

The BMI corresponding to the date of the blood sample was not always available from the request, and where necessary this was collected separately. Clinic notes from medical records, clinical letters stored by the dietetic department and a dietetic database were all accessed. These sources were also used to establish the diabetic status of patients. BMI data recording within 4 weeks from the date of the metabolic profile taken were included in the study.

The metabolic profiles and BMI data were recorded onto a single spreadsheet. Patients were excluded from the dataset for the following reasons:

1. There was a pre-existing diagnosis of diabetes mellitus.
2. The venous samples of the metabolic profile were not fasting samples.

The metabolic profiles of patients with pre-existing diabetes may have been affected by their treatment. It would not have been possible to obtain data for fasting glucose from the non-fasting samples.

The small numbers are partly explained by the difficulties encountered in the practical execution of this project. It was planned to be a prospective study, with data collection coinciding with sample collection. Sticky labels were made by the project supervisor, with the intention of collecting BMI data at the time of venepuncture. However, the use of these labels was poorly adhered to, and the BMIs of the patients were not input into the database. This necessitated retrospective collection of BMI data from several sources, including an electronic document store, a dietician's database, clinic letters and case-notes. An additional issue was that the proportion of patients with prior DM was larger than anticipated, therefore requiring exclusion and resulting in smaller numbers. The small sample size means there is a possibility that these results are unreliable.

The finalised group of subjects were categorised into diabetes, intermediate fasting glucose/pre-diabetes, and normal fasting glucose/normal HbA1c, according to three separate sets of criteria:

(i) Fasting glucose criteria as recommended by the World Health Organisation:

- DM: ≥7.0mmol/L
- IFG: 6.1-6.9 mmol/L
- Normal: ≤6.0 mmol/L

(ii) Glycated haemoglobin as recommended by the American Diabetic Association (ADA):

- DM: ≥6.5%
- Pre-diabetes: 5.7-6.4 %
- Normal: ≤5.6 %

(iii) Glycated haemoglobin as recommended by an International Expert Committee convened in 2008:

- DM: ≥6.5%
- Pre-diabetes: 6.0-6.4%
- Normal: ≤5.9 %

Body mass index was calculated from the patients' weight (in kilograms) divided by their height (in meters) squared. These measurements were taken on the date of the clinic.

Venous blood was collected into Vacutainer tubes (Becton Dickinson, Cowley, UK) and delivered to the laboratory for testing. Glucose, glycated haemoglobin (HbA1c), insulin, triglyceride, cholesterol, uric acid, alanine aminotransferase (ALT), alkaline phosphatase, sex hormone-binding globulin (SHBG) and testosterone were measured. All the metabolic profiles were done according to the routine protocol weight management clinic.

Glucose, cholesterol, triglycerides, uric acid, ALT and alkaline phosphatase were measured on a Cobas Integra 700 analyser (Roche Diagnostics, West Sussex, UK). HbA1c was measured using a Menarini Biomen HA 8140 analyser (Menarini Ltd, Berkshire, UK). Insulin and SHBG were measured by immunometric assay on a DPC Immulite analyser (Diagnostic Products Corporation, California, USA).

Data analysis was performed using IBM software package for statistical analysis (SPSS) 19 (International Business Machines Corporation, New York, USA). Descriptive statistics on baseline data (eg sex, age, BMI and metabolic profiles) were established. The F statistics of analysis of normal variance (ANOVA) was used to compare the metabolic profiles in the groups (diabetes, impaired fasting glycaemia/pre-diabetes, and normal fasting glucose/normal HbA1c) identified with the three different sets of criteria outlined above. This compared the in-between group (DM/IFG/normal) and within group (DM/IFG/normal) variance in

the metabolic profile identified by each group (ie FPG/HbA1c (IEC)/HbA1c (ADA) standardisation. Insulin, triglyceride, cholesterol ratio, uric acid, ALT, alkaline phosphatase, sex hormone binding globulin (SHBG) and testosterone were compared. ROC curve was also used to compare the metabolic profiles in the groups. Normality for the dataset was not accessed.

These data were re-analysed, separating female and male subjects, using a statistical package, PSPP (GNU project, 2014). Q-Q (quantile-quantile) plots were used to assess normality of the data. As the Q-Q plots demonstrated the data not to be normally distributed, ROC curves were used to assess the metabolic data as a non-parametric test.

The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program established criteria for the diagnosis of metabolic syndrome (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001). This recognised the importance of insulin resistance, its consequences and the associated risk of cardiovascular disease. The ATP III criteria for diagnosing the metabolic syndrome are as follows (National Heart and Lung and Blood Institue, 2002):

- Abdominal obesity
    - Men: waist circumference >40 inches (102cm)
    - Women: waist circumference >35 inches (89cm)
- Fasting glucose ≥110 to < 126mg/dL (≥6.1 to ≤7.0 mmol/L)
- Blood pressure ≥ 130/80mmHg
- Triglycerides ≥ 150mg/dl (1.7 mmol/L)
- HDL Cholesterol
    - Men: <40mg/dl (1.04mmol/L)
    - Women: <50mg/dL (1.3 mmol/L)

Three or more of these five criteria met in an individual indicates metabolic syndrome.

The assembled dataset contained fasting glucose, triglyceride and HDL cholesterol concentrations. Waist circumference data were not available. Every patient in this study had a BMI of at least 35kg/m^2; data from Lean *et al* indicates that nearly everyone with BMI in this range meets the ATP III waist circumference criterion therefore it was therefore assumed that the ATP III waist circumference criterion was met in each case (Lean *et al*., 1995). Blood pressure was not available. Patients were categorised into diabetic/prediabetic/normal groups as described earlier, using the three separate sets of diagnostic criteria outlined above. The number of ATP criteria met in the different groups was

recorded, and the number of patients who met the ATP III criteria for metabolic syndrome in each group was also recorded.

CHAPTER 4

RESULTS

4.1 Baseline Data

212 patients were included in the study. Samples excluded were: non-fasting samples (27) and samples from patients with existing diagnosis of diabetes (59), resulting in a final sample size of 126 patients. The female to male ratio was 99:27, with 120 fasting glucose samples and 117 HbA1c samples available for this study. The baseline data for the patients in the study are as follows (mean ± SD): age 43.91 ± 11.66 years, BMI 48.27 ± 7.97 kg/m^2, fasting glucose 5.42 ± 0.86 mmol/l and HbA1c 5.65 ± 0.57%.

The number of patients, age, sex and BMI classified by FPG, HbA1c (IEC standard) and HbA1c (ADA standard) are shown in table 1.

Table 1: Patient's demographics in categories classified by fasting glucose, HbA1c IEC and ADA criteria: patients' mean age, sex (male to female ratios) and mean BMI

	Fasting glucose	**HbA1c (IEC)**	**HbA1c (ADA)**
Diabetes			
n	n=5	n=7	n=7
mean age ± (SD)	47.20 Yrs ± (16.45)	44.29 Yrs ± (10.80)	44.29 Yrs ± (10.80)
male:female	1:4	0:7	0:7
mean BMI ± (SD)	48.69 kg/m^2± (7.75)	50.09 kg/m^2± (7.23)	50.09 kg/m^2± (7.23)
Mean FPG ± (SD)	8.28± (1.29)	7.2± (1.87)	7.2± (1.87)
IFG/pre-diabetes			
n	n=13	n=21	n=41
mean age ± (SD)	44.30 Yrs ± (7.81)	45.80 Yrs ± (9.85)	46.78 Yrs ± (10.90)
male:female	1:12	4:17	9:32
mean BMI ± (SD)	50.17 kg/m^2± (8.69)	48.09 kg/m^2± (7.04)	47.58 kg/m^2 ± (6.90)
Mean FPG ± (SD)	6.37± (0.23)	5.58 ± (0.69)	5.6± (0.68)
Normal			
n	n=102	n=89	n=69
mean age ± (SD)	43.28 Yrs ± (11.91)	42.92 Yrs ± (12.23)	41.51 Yrs ± (12.00)
male:female	23:79	20:69	15:54
mean BMI ± (SD)	47.70kg/m^2± (7.88)	48.13 kg/m^2± (8.21)	48.45kg/m^2 ± (8.58)
Mean FPG ± (SD)	5.16± (0.46)	5.23± (0.61)	5.11± (0.54)

The mean age of patients identified by all the methods was in the fifth decade of their lives. Male individuals were under-represented across the groups (see tables 1), reflecting referral patterns from primary to secondary (specialist) care. The mean BMI of the patients in all three groups was above 40 kg/m^2, indicating that all patients had class III obesity in this study.

Fewer people were identified as having either diabetes or IFG when FPG criteria were applied, compared with HbA1c criteria (either IEC or ADA); correspondingly more were identified as normal (See table 2). Application of HbA1c ADA criteria predictably identified more subjects as having pre-diabetes than application of HbA1c IEC criteria; it has a lower cut-off (5.7% compared with 6.0%).

Table 2: Descriptive Statistic of the Percentages of Patients Classified into Diabetes, IFG/Pre-Diabetes and Normal Groups Using Different Classifications (FPG/ HbA1c IEC's and ADA's HbA1c Criteria)

	Normal	IFG	DM
FPG	102 (85%)	13 (10.8%)	5 (4.2%)
HbA1c (IEC)	89 (76.1%)	21 (17.9%)	7 (6.0%)
HbA1c (ADA)	69 (59.0%)	41 (35.0%)	7 (6.0%)

Table 3: Number of patients co-responding between each group (Normal, Pre-DM/IFG and DM) by HbA1c (IEC and ADA) comparing to FPG categorisations

	IEC			ADA		
	Normal	Pre-DM	DM	Normal	Pre-DM	DM
FPG Categories						
Normal	80/102	14/102	3/102	64/102	30/102	3/102
IFG	7/13	4/13	1/13	4/13	7/13	1/13
Diabetes	1/5	1/5	3/5	0/5	2/5	3/5

The number of subjects with normal HbA1c, identified by the IEC criteria, correlated better with the number of normal glycaemia subjects diagnosed by FPG, compared with using the ADA criteria. More subjects in the ADA category have been identified as pre-diabetes (see tables 1 and 2). Three subjects identified with normal glycaemia in the FPG group were classified as diabetes with their HbA1c level. This finding was supported by both IEC and ADA criteria.

A larger number of subjects were identified with pre-diabetes by both IEC (n=21; 17.9%) and ADA (n=41; 35%) categorisations (see table 2). In the FPG category this correlates with 30.8% (n=4) and 53.8% (n=7) according to the IEC and ADA respectively (see table 3).

3 out of 5 subjects in the DM group identified by FPG corresponded in all 3 categorisations (table 3). One patient has been identified as DM by FPG has normal HbA1c, and one was identified as pre-DM, identified by IEC criteria. 2 out of 5 patients identified as DM by FPG has been identified as pre-DM by HbA1c ADA categorisation.

The mean metabolic profile is demonstrated in table 4.

Table 4: Mean Metabolic Profile

		FPG± SD	IEC± SD	ADA± SD
Insulin (5 - 25 µU/mL)	Normal	24.40 ± 20.57	25.12 ± 21.25	22.39 ± 18.06
	Intermediate Glycaemia	29.69 ± 15.90	27.95 ± 16.20	31.25 ± 23.03
	Diabetes	28.60 ± 29.94	21.29 ± 25.40	21.29 ± 25.40
Triglyceride (mmol/l)$^{+\infty}$	Normal	1.59 ± 0.80	1.45 ± 0.60	1.37 ± 0.57
	Intermediate Glycaemia	2.05 ± 0.87	2.22 ± 1.19	1.97 ± 0.99
	Diabetes	1.72 ± 1.19	1.89 ± 0.88	1.89 ± 0.88
Cholesterol Ratio$^{\infty}$	Normal	4.27 ± 1.35	4.20 ± 1.36	4.01 ± 1.24
	Intermediate Glycaemia	4.69 ± 1.14	4.57 ± 1.19	4.71 ± 1.40
	Diabetes	3.72 ± 0.73	4.39 ± 1.00	4.39 ± 1.00
Uric Acid (umol/l)	Normal	0.38 ± 0.09	0.38 ± 0.09	0.38 ± 0.09

	Intermediate Glycaemia	0.36 ± 0.08	0.39 ± 0.08	0.38 ± 0.09
	Diabetes	0.38 ± 0.07	0.37 ± 0.06	0.37 ± 0.06
Alanine Aminotransferase (u/l)	Normal	32.76 ± 23.11	32.65 ± 23.33	31.09 ± 22.27
	Intermediate Glycaemia	29.83 ± 17.13	36.20 ± 21.38	37.13 ± 23.84
	Diabetes	31.40 ± 9.24	36.86 ± 9.41	36.86 ± 9.41
Alkaline Phosphatase (u/l) $*^{+\infty}$	Normal	79.16 ± 21.76	77.02 ± 21.86	76.36 ± 21.43
	Intermediate Glycaemia	76.25 ± 25.83	89.65 ± 25.88	84.48 ± 25.07
	Diabetes	128.20 ± 65.19	112.71 ± 55.20	112.71 ± 55.20
Sex Hormone-Binding Globulin (nmol/l)	Normal	35.48 ± 24.50	37.02 ± 25.61	38.03 ± 28.38
	Intermediate Glycaemia	31.10 ± 14.13	25.92 ± 11.92	29.65 ± 12.32
	Diabetes	33.50 ± 18.36	27.00 ± 11.87	27.00 ± 11.87

Testosterone (nmol/L)	Normal	3.07 ± 4.13	2.99 ± 4.12	2.99 ± 4.13
	Intermediate Glycaemia	1.32 ± 1.90	2.19 ± 3.02	2.58 ± 3.63
	Diabetes	1.34 ± 0.55	1.60 ± 0.21	1.60 ± 0.21

* Significance shown in FPG; $^{+}$ significance shown in HbA1c (IEC); $^{\infty}$ significance shown in HbA1c (ADA) with the one-way ANOVA

4.2 One Way ANOVA/ Receiver Operating Characteristic (ROC)

One way ANOVA (see tables 5) and ROC curve (see tables 6, 7 and 8) were used to compare the differences of metabolic profile between the patients in the different groups.

Table 5: ANOVA F-Statistics of Metabolic Profile Identified by FPG, HbA1c IEC and ADA Categories (normal glycaemia v IFG/pre-diabetes v DM)

Parameter	FPG F Value	HbA1c (IEC) F Value	HbA1c (ADA) F Value
Insulin	0.457	0.297	2.553
Triglyceride	1.839	9.242***	8.151**
Cholesterol Ratio	1.037	0.679	3.722*
Uric Acid	0.34	0.215	0.033
Alanine Aminotransferase	0.098	0.286	1.008

Alkaline Phosphatase	9.45***	7.679***	6.866**
Sex Hormone-Binding Globulin	0.211	2.325	2.046
Testosterone	1.552	0.66	0.437

*= statistical significant (p<0.05)
**=highly statistical significant (p<0.01)
***=very highly statistical significant (p<0.001)

Parametric testing using the one-way ANOVA demonstrated the following (see table 5):

- In subjects identified by FPG, the resulting F statistics in alkaline phosphatase was high (F=9.45) and it was found to be very highly significant (p <0.001).

- In subjects identified by HbA1c (IEC standard), the resulting F statistics in triglyceride and alkaline phosphatase were high (F=9.24 and 7.68) and they were found to be very highly significant (p<0.001).

- In subjects identified by HbA1c (ADA standard), the resulting F statistics in triglyceride level was high (F=8.15) and it was found to be very highly significant (p<0.001); the resulting F statistics in alkaline phosphatase (F=6.87) was found to be highly significant (p<0.001) and the resulting F statistics in cholesterol level (F=3.722) was found to be significant (p=0.027).

Table 6: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by FPG

Area Under the Curve-FPG

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.625	.076	.102	.476	.773
TRIG	.646	.075	.056	.499	.793
CHOL	.654	.078	.044	.501	.807
CHR	.560	.070	.433	.422	.698
URIC	.453	.069	.536	.317	.589
ALT	.501	.071	.987	.361	.641
AP	.564	.088	.404	.391	.736
SHBG	.448	.078	.498	.295	.601
TEST	.416	.064	.268	.289	.542

Table 7: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (IEC)

Area Under the Curve-HbA1c (IEC)

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.556	.063	.392	.432	.681
TRIG	.704	.063	.002	.580	.828
CHOL	.617	.064	.075	.491	.744
CHR	.582	.060	.215	.464	.699
URIC	.560	.059	.366	.444	.675
ALT	.639	.055	.034	.532	.747
AP	.675	.066	.008	.546	.805
SHBG	.316	.060	.005	.199	.434
TEST	.540	.063	.546	.417	.662

Table 8: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (ADA)

Area Under the Curve-HbA1c (ADA)

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.641	.053	.012	.538	.744
TRIG	.698	.051	.000	.598	.797
CHOL	.617	.054	.036	.512	.723
CHR	.636	.052	.015	.535	.738
URIC	.512	.055	.829	.405	.620
ALT	.609	.054	.051	.504	.714
AP	.618	.055	.035	.509	.726
SHBG	.415	.054	.129	.309	.522
TEST	.536	.056	.523	.426	.645

A non-parametric test, Receiver Operating Characteristic, ROC (see tables 6-8), was used to compare the normal (glycaemic/HbA1c) groups with IFG/pre-diabetes and the DM group. It demonstrated the following:

- In subjects identified by HbA1c (IEC standard) ROC curves of triglyceride, alkaline phosphatase and sex hormone-binding were highly significant ($p<0.01$). In addition, the ROC curve of alanine aminotransferase was significant ($p=0.034$).

- In subjects identified by HbA1c (ADA standard), the ROC curve of triglyceride level are found to be very highly significant ($p<0.001$), the ROC curve of in alkaline phosphatase and cholesterol ratio are found to be

significant. The ROC curve of insulin was also shown to be significant ($p<0.05$).

Subjects were separated into male and female and the metabolic profile was re-analysed. Data were not normally distributed (see Appendix 4: Q-Q Plots), therefore ROC was used to analyse data with a non-normal disturbution (see table 9-14).

Table 9: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by FPG (Female)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.32	.11	.395	.14	.49
TRIG	.43	.13	.764	.22	.65
CHR	.79	.08	.176	.66	.93
URIC	.95	.05	.040	.87	1.02
ALT	.62	.25	.582	.21	1.03
AP	.78	.12	.193	.59	.98
SHBG	.30	.15	.367	.06	.55
TEST	.83	.14	.133	.60	1.05

Table 10: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by FPG (Males)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.38	.08	.141	.24	.52
TRIG	.34	.08	.045	.20	.47
CHR	.40	.08	.213	.27	.52
URIC	.45	.08	.548	.32	.58
ALT	.46	.08	.611	.33	.58
AP	.39	.09	.187	.24	.54
SHBG	.59	.08	.294	.45	.72
TEST	.47	.08	.685	.34	.59

Table 11: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (IEC) (Female)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.23	.12	.096	.03	.43
TRIG	.21	.14	.075	-.02	.45
CHR	.26	.12	.141	.06	.46
URIC	.74	.13	.141	.52	.95
ALT	.59	.17	.588	.31	.86
AP	.23	.16	.088	-.04	.49
SHBG	.44	.14	.699	.21	.66
TEST	.69	.16	.245	.43	.95

Table 12: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (IEC) (Male)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.49	.07	.876	.37	.60
TRIG	.31	.07	.010	.20	.43
CHR	.44	.07	.428	.33	.55
URIC	.35	.06	.038	.24	.45
ALT	.30	.06	.006	.21	.39
AP	.35	.07	.038	.23	.47
SHBG	.73	.06	.001	.63	.84
TEST	.39	.07	.120	.26	.51

Table 13: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (ADA) (Female)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.27	.10	.069	.10	.45
TRIG	.16	.08	.006	.02	.29
CHR	.37	.12	.297	.18	.56
URIC	.63	.13	.283	.42	.85
ALT	.70	.11	.114	.51	.88
AP	.34	.12	.200	.14	.55
SHBG	.33	.12	.180	.14	.52
TEST	.63	.12	.297	.43	.83

Table 14: Comparison of Metabolic Profile (Normal vs IFG/pre-diabetes and DM combined) Identified by HbA1c (ADA) (Male)

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.39	.06	.072	.29	.49
TRIG	.34	.06	.013	.25	.44
CHR	.36	.06	.027	.26	.46
URIC	.42	.06	.217	.32	.52
ALT	.31	.06	.003	.22	.41
AP	.39	.06	.090	.29	.50
SHBG	.64	.06	.027	.54	.74
TEST	.39	.06	.079	.29	.49

- In male subjects identified by FPG, ROC curve of triglyceride was statistical significant ($p < 0.05$).

- In male subjects identified by HbA1c (IEC standard) ROC curves of triglyceride, and alkaline phosphatase were highly significant ($p < 0.01$). In addition, the ROC curve of alanine aminotransferase and uric acid was significant ($p < 0.01$) and SHBG was very highly significant ($p = 0.001$).

- In female subjects identified by HbA1c (ADA standard), the ROC curve of triglyceride level was found to be highly significant ($p < 0.01$); in male subjects identified by HbA1c (ADA standard), the ROC curves of triglyceride, cholesterol ratio and SHBG level were found to be significant

(p <0.05) and the ROC curve of alanine aminotransferase was found to be highly significant (p <0.01).

4.3 ATP Criteria for Metabolic Syndrome

The following tables show the number of subjects meeting each of the ATP III criteria in the IFG/pre-diabetes group:

Table 15: Patients meeting fasting glucose criterion of ATP III (6.1-6.9 mmol/L)

FPG	IEC	ADA
13/13	4/21	7/41

Table 16: Patients meeting triglyceride criterion of ATP III (>1.7 mmol/L)

FPG	IEC	ADA
5/13	11/21	17/41

Table 17: Patients meeting HDL cholesterol criterion of ATP III (<1.04 mmol/L for men, <1.30 mmol/L for women)

FPG	IEC	ADA
8/13	13/21	25/41

All of the subjects had BMIs over 35 kg/m^2, therefore it was assumed that the waist circumference criterion of the ATP III criteria had been met (men: >40 inches; women: >35 inches). Making this assumption, 10 out of 13 subjects (76.92%) met three out of five of the ATP III criteria using FPG; 8 out of 21 subjects (38.10%) using IEC and 14 out of 41 subjects (34.15%) using the ADA criteria in the IFG/pre-diabetes group. Blood pressure data was not transferred into the dataset therefore it was not available for the study.

CHAPTER 5

DISCUSSION

Diagnosis of DM is largely based around glycaemia. The advent of glycated haemoglobin (HbA1c) measurement in the 1970s provided an additional tool for providing more than a 'snapshot' of glycaemic control, and has been proven to be a reliable monitoring tool for diabetes (Kilpatrick *et al*., 2009); and it has also been shown to be predictive of complications (The DCCT Research Group, 1995, Gabbay *et al*., 1977, UK Prospective Diabetes Study (UKPDS) Group, 1998).

The National Diabetic Data Group in 1979 established the oral glucose tolerance test as the preferred diagnostic test for DM (National Diabetes Data Group, 1979). However, it has poor reproducibility and is a weaker indicator of long-term complications compared with other measures of hyperglycaemia (McCance *et al*., 1997). OGTT involves measuring blood glucose values while fasting and at 2 hours after a 75g oral glucose load is consumed (Scottish Intercollegiate Guidelines Network, 2010). Both OGTT and FPG require fasting. OGTT is recommended if casual blood glucose results lie in an uncertain range and if fasting glucose results are below those which establish the diagnosis of diabetes (Scottish Intercollegiate Guidelines Network, 2010).

FPG directly measures the concentration of glucose which contributes to some of the complications in DM (The DCCT Research Group, 1995, Gabbay *et al.*, 1977, UK Prospective Diabetes Study (UKPDS) Group, 1998). However, the fasting requirement can be a challenge for some patients.

In comparison, measurement of HbA1c does not require fasting and is therefore a clinically easier test to perform. The DCCT and UKPDS studies showed a correlation between HbA1c and macrovascular and microvascular complications. HbA1c is an established test for monitoring DM. It has lower within-person variability than glucose and it has been considered as a diagnostic test. If specific complications of diabetes are caused by chronic hyperglycaemia, long-term glycaemic exposure (eg HbA1c) should provide a better marker for the presence and severity of DM (The International Expert Committee, 2009).

The potential utility of HbA1c in diabetes was first mentioned in 1985 (World Health Organisation, 1985). In 1997, the expert committee on the Diagnosis and Classification of Diabetes Mellitus (The International Expert Commitee, 1997) re-examined the basis of diagnosing DM, based on the relationship between glucose and the presence of long-term complications as the basis for the diagnosis of DM and they summarised the data negating the widespread hypothesis that the OGTT was the gold standard for the diagnosis of DM (IEC 2009). Based on cross-sectional epidemiological studies of three populations

(Egyptian, n=1018; Pima Indians n=960 and NHANES n=2821) (McCance *et al*., 1994, Engelgau *et al*., 1997, The International Expert Committee, 2009), each examined glycaemia as FPG, OGTT and HbA1c with retinopathy via fundus photography or direct ophthalmoscopy. A clear relationship was found between glycaemia and the risk of retinopathy. The first expert committee on the Diagnosis and Classification of Diabetes Mellitus revised the diagnostic criteria, using the association between FPG levels and the presence of retinopathy to identify the threshold glucose concentration (ADA, 1997). The previous FPG cut-point of 140mg/dl (7.8mmol/l) was substantially above the glucose concentration at which the prevalence of retinopathy begin to increase, therefore the committee recommended that the FPG cut-point be decreased to ≥126mg/dl (7.0 mmol/l). The new fasting cut-point represents a degree of hyperglycaemia similar to the OGTT 2-hour glucose concentration of 11.1 mmol/L and the diagnosis with either measure results in a similar prevalence of diabetes in the population (The International Expert Committee, 2009).

HbA1c was introduced into clinical use in the 1980s and has become a corner-stone of clinical practice (Massi-Benedetti, 2006). The 1997 IEC committee report showed the prevalence of retinopathy increases substantially at HbA1c values starting between 6-7% (American Diabetes Association, 1997). An analysis derived from DETECT-2 study and including the previous 3 studies mentioned, diabetes specific ‘moderate’ retinopathy was virtually non-existent in the >20000 subjects who had HbA1c <6.5% involved in the study (Colagiuri *et*

al., 2011). However, at that point it was recommended against using HbA1c to diagnose diabetes due to the difficulties with assay standardisation (American Diabetes Association, 1997).

The WHO report in 1999 responded to the ADA recommendations, and the diagnostic thresholds of FPG were changed according to their recommendations. The standards employed by the WHO are mostly used worldwide. The IEC and Diabetes UK also are aligned with these recommendations.

In 2003 follow-up report from the IEC noted that, while the National Glycohaemoglobin Standardisation Program had successfully standardised the majority of assays in the US (Little *et al*., 2001), the use of HbA1c was still not recommended due to the disadvantages. The WHO 2005 consultation also stated that HbA1c should not be used as a diagnostic test, as challenges of measurement accuracy outweighed the convenience of its use (World Health Organisation, 2006).

In the 2009 report, the IEC endorsed the use of HbA1c test to diagnose diabetes, with a threshold of over 6.5%. In selecting a diagnostic level of over 6.5%, the IEC balanced the stigma and the cost of mistakenly identifying individuals as diabetic, against the minimal clinical consequences of delaying the diagnosis in

someone with an HbA1c level of over 6.5% and therefore they have agreed to emphasise specificity (ability correctly to identify true negatives, i.e. people without diabetes) rather than sensitivity (ability correctly to identify true positives, i.e. people with diabetes). This decision was aided by the decision to recommend effective prevention strategies for the at-risk group with HbA1c between 6-6.5% (The International Expert Committee, 2009). This range should not be an absolute threshold at which preventive measures are initiated (Tuomilehto *et al*., 2001, Diabetes Prevention Program Research Group, 2002). When assessing risk and implementing strategies, other DM risks should be taken into account (The International Expert Committee, 2009). The IEC has also stated the FPG concentration, rather than the OGTT, is the preferred test to diagnose DM as it is more convenient, less costly and time-consuming, and reproducibility of the test is superior (The International Expert Committee, 2009).

When recommending the use of HbA1c to diagnose diabetes in the 2009 report (The International Expert Committee, 2009), the IEC stressed that those with HbA1c above normal (6.0%) but below the diagnostic threshold (6.5%) are at high risk of developing diabetes (the incidence of diabetes is increased tenfold in this range than when HbA1c is <6.0% (American Diabetes Association, 2010). The National Health and Nutrition Examination Survey (NHANES) indicated that people with HbA1c 5.5-6% have a 5-year of cumulative incidence of diabetes of 12-25%. A large prospective study found that a cut-off point of 5.7% has a sensitivity of 66% and specificity of 88% in the identification of subsequent 6 year

diabetes incidence (Droumaguet C, 2006). The ADA had recommended individuals with an HbA1c of 5.7-6.4% should be informed of their increased risk for diabetes as well as cardiovascular disease and counselled about effective strategies, such as weight loss and physical activity (American Diabetes Association, 2010).

When the current study was planned in 2009, HbA1c was routinely used to monitor glycaemic control in DM but not for diagnostic purposes. However, the WHO 2011 recommendation stated HbA1c can be used as a test in the diagnosis of DM, providing that 'stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values' and 'there are no conditions present to preclude its accurate measurement' (WHO 2011). An HbA1c of 6.5% is recommended as the cut-off point for the diagnosis of diabetes. This was supported by a systematic review (World Health Organisation, 2011a) conducted by the Boden Institute of Obesity, Nutrition and Exercise, Sydney. The A1c Derived Average Glucose (ADAG) study established a validated relationship between HbA1c and average glucose across patient populations (Nathan, 2008). An HbA1c of <6.5% does not exclude DM diagnosed using glucose tests (WHO 2011) and the consultation concluded that there is insufficient evidence to make any formal recommendation on the interpretation of HbA1c levels below 6.5% (WHO 2011).

A study was conducted to compare diabetes, IFG/pre-diabetes and normal glycaemic patients identified by OGTT and the proposed criteria (HbA1c>6.5% for diabetes, HbA1c 6-6.4% (IEC) and HbA1c 5.7-6.4% (ADA) for IFG/pre-diabetes) in three datasets: the Prospective Screening for Impaired Glucose Study, the NHANES III and NHANES 2005-2006 (Olson *et al*., 2010). Using OGTT, 5.8% of the combined study subjects had new diabetes, 36% had pre-diabetes and 58% had normal glucose tolerance. By the IEC criteria, 2.2% of patients were classified as having diabetes, 6.2% as high risk and 91.8% had normal glucose tolerance; 2.2% were classified as diabetes and 19.3% were high risk and 77.8 had normal glucose tolerance by ADA criteria. Evidence has revealed both the HbA1c criteria resulted in more normal diagnosis and ADA criteria resulted in a distribution of diagnosis more similar to those with OGTT criteria, by identifying more patients as high risks and fewer as normal glucose tolerance (Olson *et al*., 2010).

In the current study, we wished to examine a group of patients who are at elevated risk of DM on account of their body mass index (BMI). Specifically, we wished to establish if the application of these different categorisations identified the same subjects in each corresponding group, or different ones. We also wished to characterise the metabolic profiles of the patients identified in each group, as a secondary outcome. Therefore in this study we have compared the number of patients categorised as diabetic/pre-diabetic/normal, and their

metabolic characteristics, using the following: (1) FPG (2) HbA1c categorised according to IEC criteria: (3) HbA1c categorised according to ADA criteria.

Until June 2009 HbA1c were reported in Diabetes Control and Complication Trial (DCCT)-aligned format, with the units being the proportion of the total haemoglobin that is glycated expressed as a percentage (Scottish Intercollegiate Guidelines Network, 2010). A new standard published by the International Federation of Clinical Chemistry and Laboratory Medicine reports results in mmol/mol (Barth *et al*., 2008). In this study the values of HbA1c were written as percentages (%), rather than the new unit in milli-moles per mol (mmol/mol). This is because many of the specimens were collected prior to the advent of dual reporting of old and new units. It was also anticipated that comparisons might be drawn with existing (older) studies also reporting HbA1c as percentages.

In our study there were 99 females and 27 male patients, and the female preponderance was observed in all groups established by each diagnostic system (FPG/HbA1c-IEC/Hb1c-ADA). The patient cohort was recruited specifically from a specialist obesity service. The incidence of obesity has been reported to be greater in men than women (Kopelman *et al.*, 2010). It is likely that women are more likely to seek help regarding to obesity (Hebebrand, 2009) and other health problems, therefore they present to their general practitioners and are more likely to be referred to the obesity clinic to address the obesity problem.

The percentage of patients identified in each group by different categorisations are different, with the exception of the diabetes group identified by HbA1c (both IEC and ADA standards), as they share the same criteria for DM patient (HbA1c >6.4%). The numbers identified as diabetic by each system were small and not amenable to statistical analysis. The key difference observed was that more patients were identified in the intermediate category by the HbA1c ADA system than by either of the other two systems, and correspondingly fewer patients as normoglycaemic. More patients have been identified as normoglycaemic by the FPG categorisation.

Cross tabulisation was carried out to compare the number of patients identified in each group categorised by HbA1c (both IEC and ADA) categorisation and FPG. HbA1c (IEC categorisation) agreed more with the FPG categorisation, in relation to the number of subjects identified as normal HbA1c/ normal glycaemia. This is likely due to a lower threshold the ADA classification has for pre-DM (5.7% as to 6.0%). Therefore, some subjects were categorised as pre-diabetic by the ADA criterion but as normoglycaemic by other diagnostic systems. Subjects could be identified as intermediate glycaemia but with a normal HbA1c.

Ethnic differences could not be demonstrated, as most patients in our cohort were Caucasian. Caution is advised in interpretation of these results due to the small sample size.

Metabolic syndrome is a complex disorder defined by factors, which increase the risk of cardiovascular atherosclerotic diseases and diabetes mellitus type 2. It was described by Reaven (1998) as Syndrome X. He noted that several risk factors, e.g. dyslipidemia, hypertension, hyperglycaemia, are clustered together; and he postulated that insulin resistance was the underlying factor associated with diabetes and coronary heart disease (Reaven, 1988). Different organisations, for example, the WHO and the American Association of Clinical Endocrinologists, proposed different clinical criteria for metabolic syndrome (Grundy 2004, WHO 1998, AACE). The National Cholesterol Education Program's Adult treatment Panel III report (ATP III) identified cardiovascular disease as the primary clinical outcome of metabolic syndrome; most people with metabolic syndrome have insulin resistance and increased risks of diabetes. The ATP III identified 6 components of the metabolic syndrome:

1. Abdominal obesity
2. Atherogenic dyslipidemia
3. Hypertension

4. Insulin resistance +/- glucose intolerance
5. Proinflammatory state
6. Prothrombotic state

The pathogenesis of metabolic syndrome involves:

- Obesity and abnormal body fat distribution: abdominal obesity especially correlates with metabolic risk factors. Products released by adipose tissue including non-esterified fatty acids (NEFA), cytokines, plasminogen activator inhibitor-1 (PAI-1) and adiponectin, cause insulin resistance, a proinflammatory and prothrombotic state and worsening metabolic risk factors. The strong connection between obesity and these risk factors defined metabolic syndrome as a clustering of metabolic complications of obesity (Grundy *et al*., 2004).
- Insulin resistance-fatty liver and atherogeneic dyslipidaemia can be caused by insulin resistance: enhanced release of NEFA from insulin-resistant adipose tissue leads to a liver overloaded with lipids, leading in turn to increase hepatic output of very-low-density lipoprotein particles carrying predominantly triglycerides. Insulin resistance in muscles predisposes glucose intolerance, worsened by increased hepatic gluconeogenesis in insulin-resistant liver (Grundy *et al*., 2004).

- Independent and other contributing factors including genetic and other acquired factors, advanced age and endocrine factors. These factors can affect lipoprotein metabolism, blood pressure regulation, body fat distribution and pathogenesis (Grundy *et al*., 2004).

Using one way-ANOVA, we compared the metabolic profiles of patients categorised according to each of the three diagnostic tests. The metabolic profile included insulin, cholesterol ratio, triglyceride, uric acid, androgen status (sex-hormone binding globulin (SHBG) and testosterone), and liver function tests (including alanine aminotransaminase and alkaline phosphatase). Differences were observed in some of the metabolic profiles of patients identified by different diagnostic systems. A high F value and a very highly statistical significance in alkaline phosphatase of patients identified by all the different categorisations was demonstrated by FPG categorisation; this could be due to patients with impaired fasting glycaemia and diabetes having higher alkaline phosphatase reflecting fatty liver. Normality of the entire data was not assessed. Differences in alkaline phosphatase and triglyceride were demonstrated in patients identified by HbA1c (IEC) and this finding was also corroborated using the ROC test.

As anticipated, mean triglycerides and total/HDL cholesterol ratios were higher in the IFG/pre-diabetes and diabetes groups identified by all diagnostic systems, comparing to normoglycaemic patients. This probably reflects metabolic

syndrome, which is known to be associated with hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia. Most people with obesity have relatively low insulin sensitivity (Bogardus *et al*., 1985), but there is variation in insulin sensitivity within the obese population (Abbasi *et al*., 2002). Certain metabolic characteristics are expected from obese patients, for examples, high insulin, decreased testosterones in males, increased testosterones in females and decreased SHBG (Pasquali, 2006).

Statistical significant in F-statistics were observed more frequently in the measured metabolic profile of patients identified by HbA1c (ADA) category (e.g. alkaline phosphatase, $p=0.02$; triglycerides $p<0.001$ and cholesterol ratio $p=0.027$). This probably reflects the wider pre-diabetic range of HbA1c (ADA).

After analysing the data with the male and female sex separately, it was found that the data was not evenly distributed; ROC was therefore used to analyse the metabolic data. There were no differences illustrated in the metabolic profile of female subjects except triglyceride in the ADA group. This probably reflects the wider pre-diabetic range of HbA1c (ADA). There were significant differences in triglycerides across the male groups in each categorisation (FPG, HbA1c IEC and ADA). Differences were also demonstrated in uric acid, alanine aminotransferase, alkaline phosphatase and SHBG in the male subjects in the male subjects of the HbA1c (IEC) categorisation; and cholesterol ratio, alanine

aminotransferase and SHBG in the male subjects of the HbA1c (ADA) categorisation. The differences might be due to the very small sample number of the male group after the splitting of the subjects into male and female. These results are to be interpreted with caution as the small number may mean the results are not representative.

This patient cohort was chosen for this study, since it is considered that obesity is associated with DM and metabolic syndrome. 59 out of the original 212 patients (27.8%) were excluded for this study due to existing DM and were on oral anti-hyperglycaemic agents, which reflected the high prevalence of diabetes in this particular patient cohort.

Patients who were identified with diabetes by HbA1c might have had been identified with IFG, or normal glycaemic by FPG test and therefore are false positives. This data is to be interpreted with caution due to the small sample size and the BMI results can be incidental.

Additional tests were carried out to establish any differences in the number of patients identified by FPG, HbA1c (IEC and ADA criteria) matching the ATP III criteria. While the blood pressure information was not available, all the patients in the groups were already assumed to have abdominal obesity due to the high BMI

(Lean *et al*., 1995). FPG identified more patients with higher fasting glucose, in the IFG/pre-diabetes category, compared to patients identified with the HbA1c categorisation. Patients who met the HDL criterion were similar in the IFG/pre-diabetes category. This could be due to obese patients having lower HDL. The differences in the number of patients with different fasting glucose and triglyceride levels might have contributed to the differences in the FPG and the HbA1c (both ADA and IEC) tests meeting the ATP III criteria for metabolic syndrome.

There are strengths of this study: the cohort of patients was recruited from a very similar background: the weight management specialist service in a single centre. The population from this cohort were similar, which means there were less discrepancies and variations in the patient's demographic and metabolic profile. Patients' age, BMI and race were similar, therefore fewer discrepancies were caused by these possible influencing factors. These patients live in a similar geographical area in Tayside and therefore the patients' data is less affected by environmental factors. As discussed earlier, one of the disadvantages of the HbA1c test is the variations in different laboratories. Because this is a single centre study, all the blood samples from biochemistry were dealt with by a single laboratory therefore the variations are limited and the results are more consistent.

The challenge of this study was that although the study was initially planned as a prospective study, some data had to be gathered retrospectively. Some of the data were missing (eg some of the FPG, HbA1c and metabolic profile measurements) therefore they were unavailable for analysis. Also, the retrospective study replied on others for accurate record-keeping eg the BMI data, which may vary from clinic to clinic.

When planning the project, past medical history and medication were not considered therefore these data were not collected. Some medications eg anti-hypertensives and statins, might have effects on the metabolic profiles (eg lipids) that affect the results of the study. As medication data were not collected, some of the diabetic subjects excluded for the study might not have been on glucose-lowering therapy therefore the number for diabetic patients could have been greater. Exclusion of the 59 subjects may mean metabolic data for the diabetic group of subject is lost, which may have been valuable for analysis.

When the metabolic profile data was first analyse as a whole set (ie with male and female subject merged in), the normality of the data was not analysed. Both ANOVA and ROC approach were used to analyse the data, to cover the data distribution, whether it is parametric or not. This can lead to interpreting the data incorrectly although some results did correlate. Some hormones, for examples, SHBG and testosterone, the levels vary between male and female. Without

separating the results into male and female, the analysis of this data would not be useful.

After separating the subjects into male and female for re-analysis of the metabolic data, some of the groups have small subject number (eg male subjects in IFG/pre-diabetes groups) and others have no male subjects in the diabetes group in both HbA1c (IEC and ADA) categorisations, leading to small samples for analyses and thus potentially inaccurate results.

Other weaknesses of the project were as follows: the comparison of the patient's group identified by FPG/ HbA1c (IFG and ADA criteria) with the ATP III criteria was a post hoc analysis (conceived after the initiation of the project; i.e. the data were not collected specifically for this analysis and therefore not all the data was available for this purpose. Blood pressure of the patients was not recorded into the database and therefore could not be used as a criterion for comparison. Waist circumference was not available in the database, however, since all the subjects had BMI greater than 35kg/m^2 it was estimated that this criterion was met (Lean *et al*., 1995).

CHAPTER 6

CONCLUSION

In our study, HbA1c (ADA) criteria predictably identified more people at risk of developing diabetes than HbA1c (IEC) criteria, on account of a lower HbA1c cut-off (5.7% versus 6.0%). It also identified more people in the pre-diabetes category than IFG were identified by FPG. In both the pre-diabetes groups identified by the HbA1c IEC and ADA criteria, the fasting glucose values are lower than the IFG category in the FPG test. The implication of this is that some people are identified as having pre-diabetes by HbA1c-based criteria, who do not have IFG. It may mean that these patients are more at risk of developing DM in the future therefore follow-ups are required. However, it could also mean that these patients could be falsely identified as pre-diabetes, or a category of 'patients in higher risks of developing DM'. Labeling patients as pre-diabetes, or indicating that they have higher risks in developing DM, may have implications in their daily living such as employment and insurance. The diagnostic tests, FPG or HbA1c, have to be interpreted in context with individual basis, along with risk factors an individual may have, in terms of developing DM.

The ANOVA test of the metabolic profile showed significant F statistics in triglyceride was also identified in HbA1c (both IEC and ADA standards) and

cholesterol ratio in the HbA1c (ADA) diagnostic test. A wider range of HbA1c in the pre-diabetes group may mean a different distribution of patients therefore the more varied metabolic profile results within and between the groups. The significance of the ROC curves in the male groups after separating the metabolic profile into female and male sexes might not be relevant, due to the small number of subjects.

There were also differences detected in the number of patients identified with IFG/pre-diabetes by all three different diagnostic systems (FPG, HbA1c IEC and ADA criteria) who met three of the ATP III criteria for metabolic syndrome. This was likely due to the lower fasting glucose observed in patients identified by HbA1c-based systems. FPG measures the molecules that directly measure glycaemia, while HbA1c measures glycated haemoglobin, which reflects chronic glycaemia and it does not measure the glucose molecules directly, but rather the relation between glycaemia and Hb glycation.

It is thus possible to have discrepancies identifying IFG and pre-diabetes using different classifications e.g. FPG and HbA1c, therefore explaining the lower fasting glucose in the HbA1c intermediate groups than the IFG group identified by FPG. Obesity correlates with metabolic risk factors therefore the reason these patients have similar metabolic profiles could be due to the pathophysiology in obesity.

This research project provided a small scale study to compare HbA1c with FPG in the diagnosis of type 2 diabetes and intermediate hyperglycaemia (impaired fasting glycaemia), in our specific cohort of patients. This study was limited by small sample size, after the exclusion of patients with existing DM and patients with non-fasting blood samples. These tests should be repeated on a larger sample size to ensure the stability of the results, and also the possibility of multi-centre studies. It is recommended that a medium to long-term follow-up of these patients is conducted in order to observe the development of diabetes from the IFG/pre-diabetes group identified by all three tests (FPG, HbA1c IEC and ADA criteria).

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APPENDIX 1: FREQUENCY TABLE

Frequency Table

FBG_group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal0	102	81.0	85.0	85.0
	Intermed Glycemia1	13	10.3	10.8	95.8
	Diabetes2	5	4.0	4.2	100.0
	Total	120	95.2	100.0	
Missing	System	6	4.8		
Total		126	100.0		

HbA1c_IEC_Group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal0	89	70.6	76.1	76.1
	Intermed Glycemia1	21	16.7	17.9	94.0
	Diabetes2	7	5.6	6.0	100.0
	Total	117	92.9	100.0	
Missing	System	9	7.1		
Total		126	100.0		

HbA1b_ADA_Group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal0	69	54.8	59.0	59.0
	Intermed Glycemia1	41	32.5	35.0	94.0
	Diabetes2	7	5.6	6.0	100.0
	Total	117	92.9	100.0	
Missing	System	9	7.1		
Total		126	100.0		

APPENDIX 2: ANOVA

ANOVA-FPG

		Sum of Squares	df	Mean Square	F	Sig.
INSU	Between Groups	385.150	2	192.575	.457	.635
	Within Groups	48934.128	116	421.846		
	Total	49319.277	118			
TRIG	Between Groups	2.481	2	1.240	1.839	.164
	Within Groups	78.914	117	.674		
	Total	81.395	119			
CHOL	Between Groups	5.997	2	2.999	2.827	.063
	Within Groups	123.038	116	1.061		
	Total	129.035	118			
CHR	Between Groups	3.591	2	1.796	1.037	.358
	Within Groups	200.860	116	1.732		
	Total	204.452	118			
URIC	Between Groups	.005	2	.003	.340	.712
	Within Groups	.928	117	.008		
	Total	.934	119			
ALT	Between Groups	97.604	2	48.802	.098	.906
	Within Groups	57509.220	116	495.769		
	Total	57606.824	118			
AP	Between Groups	11755.779	2	5877.890	9.450	.000
	Within Groups	72154.540	116	622.022		
	Total	83910.319	118			
SHBG	Between Groups	231.814	2	115.907	.211	.810
	Within Groups	64374.961	117	550.213		
	Total	64606.775	119			
TEST	Between Groups	46.619	2	23.310	1.552	.216
	Within Groups	1712.210	114	15.019		
	Total	1758.829	116			

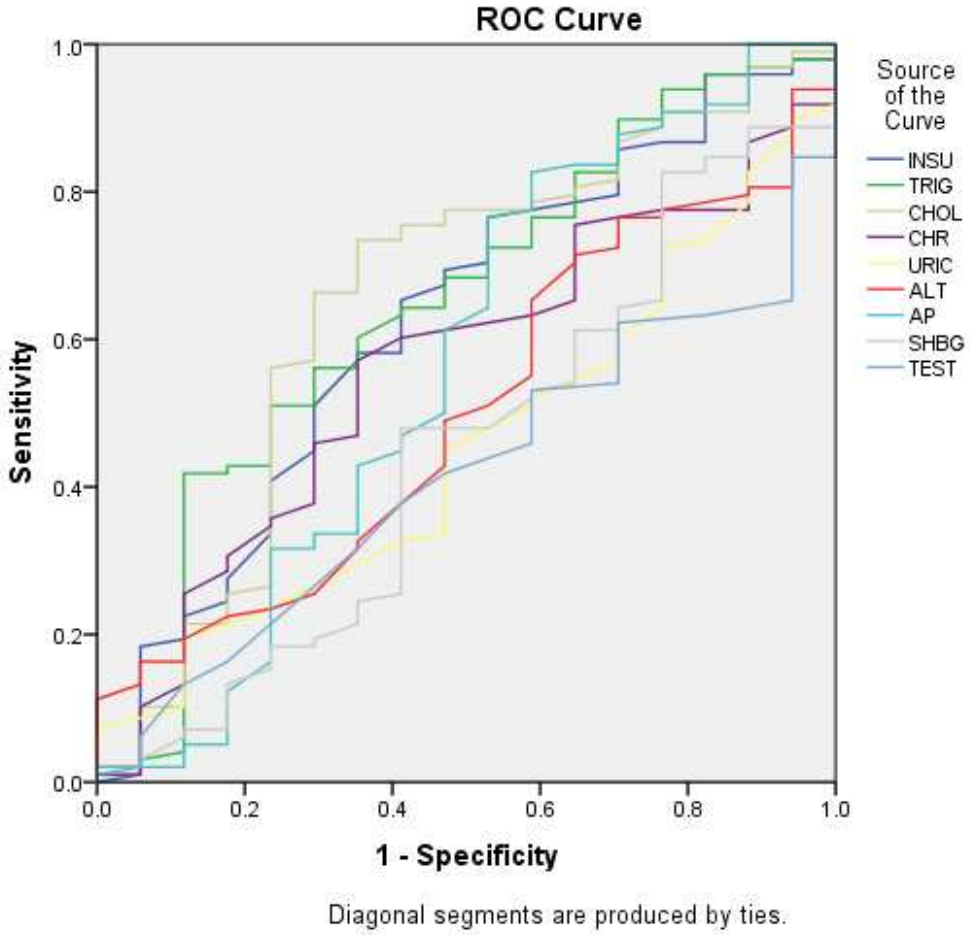
ANOVA-HbA1c (IEC)

		Sum of Squares	df	Mean Square	F	Sig.
INSU	Between Groups	255.291	2	127.646	.297	.744
	Within Groups	48588.019	113	429.982		
	Total	48843.310	115			
TRIG	Between Groups	10.515	2	5.257	9.242	.000
	Within Groups	64.853	114	.569		
	Total	75.368	116			
CHOL	Between Groups	6.362	2	3.181	3.050	.051
	Within Groups	117.851	113	1.043		
	Total	124.214	115			
CHR	Between Groups	2.357	2	1.179	.679	.509
	Within Groups	196.170	113	1.736		
	Total	198.527	115			
URIC	Between Groups	.003	2	.002	.215	.807
	Within Groups	.911	114	.008		
	Total	.915	116			
ALT	Between Groups	288.706	2	144.353	.286	.752
	Within Groups	57128.259	113	505.560		
	Total	57416.966	115			
AP	Between Groups	9930.575	2	4965.287	7.679	.001
	Within Groups	73065.934	113	646.601		
	Total	82996.509	115			
SHBG	Between Groups	2504.056	2	1252.028	2.325	.102
	Within Groups	61386.873	114	538.481		
	Total	63890.929	116			
TEST	Between Groups	19.552	2	9.776	.660	.519
	Within Groups	1643.862	111	14.810		
	Total	1663.414	113			

ANOVA-HbA1c (ADA)

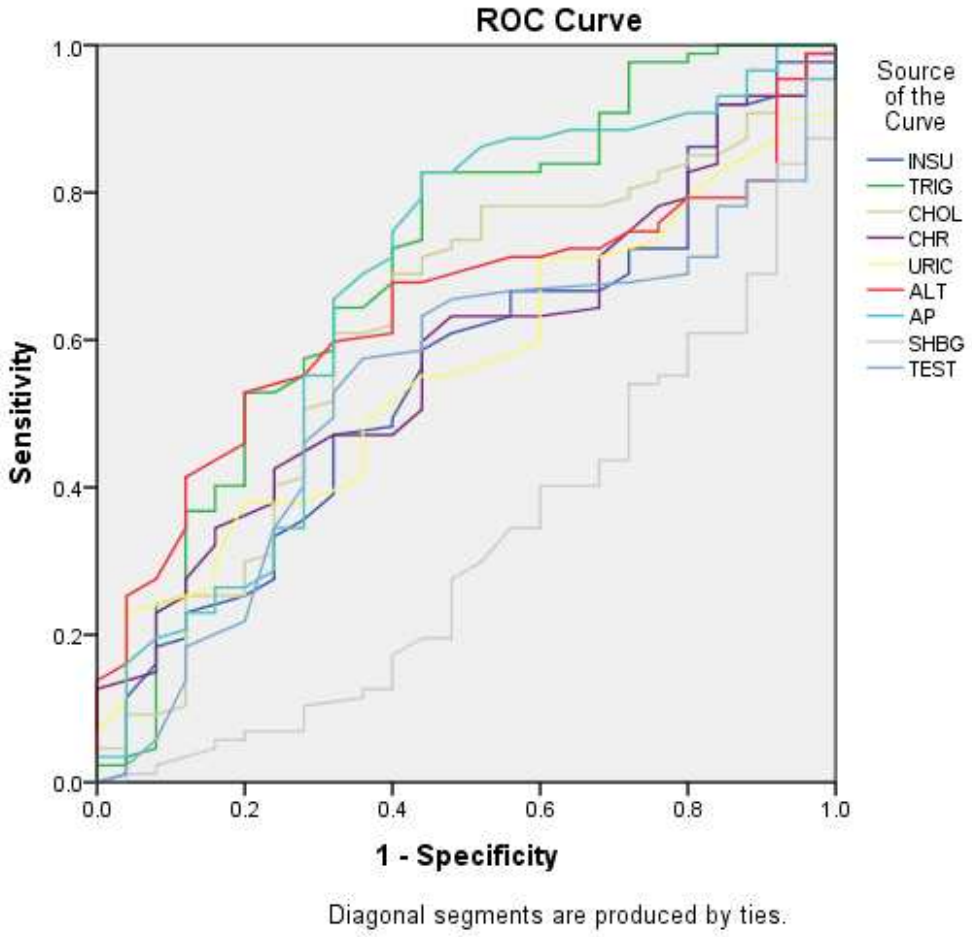
		Sum of Squares	df	Mean Square	F	Sig.
INSU	Between Groups	2111.947	2	1055.973	2.553	.082
	Within Groups	46731.363	113	413.552		
	Total	48843.310	115			
TRIG	Between Groups	9.430	2	4.715	8.151	.000
	Within Groups	65.938	114	.578		
	Total	75.368	116			
CHOL	Between Groups	7.551	2	3.776	3.657	.029
	Within Groups	116.662	113	1.032		
	Total	124.214	115			
CHR	Between Groups	12.269	2	6.134	3.722	.027
	Within Groups	186.258	113	1.648		
	Total	198.527	115			
URIC	Between Groups	.001	2	.000	.033	.967
	Within Groups	.914	114	.008		
	Total	.915	116			
ALT	Between Groups	1006.255	2	503.128	1.008	.368
	Within Groups	56410.710	113	499.210		
	Total	57416.966	115			
AP	Between Groups	8993.163	2	4496.582	6.866	.002
	Within Groups	74003.346	113	654.897		
	Total	82996.509	115			
SHBG	Between Groups	2214.319	2	1107.159	2.046	.134
	Within Groups	61676.611	114	541.023		
	Total	63890.929	116			
TEST	Between Groups	13.004	2	6.502	.437	.647
	Within Groups	1650.411	111	14.869		
	Total	1663.414	113			

APPENDIX 3: ROC



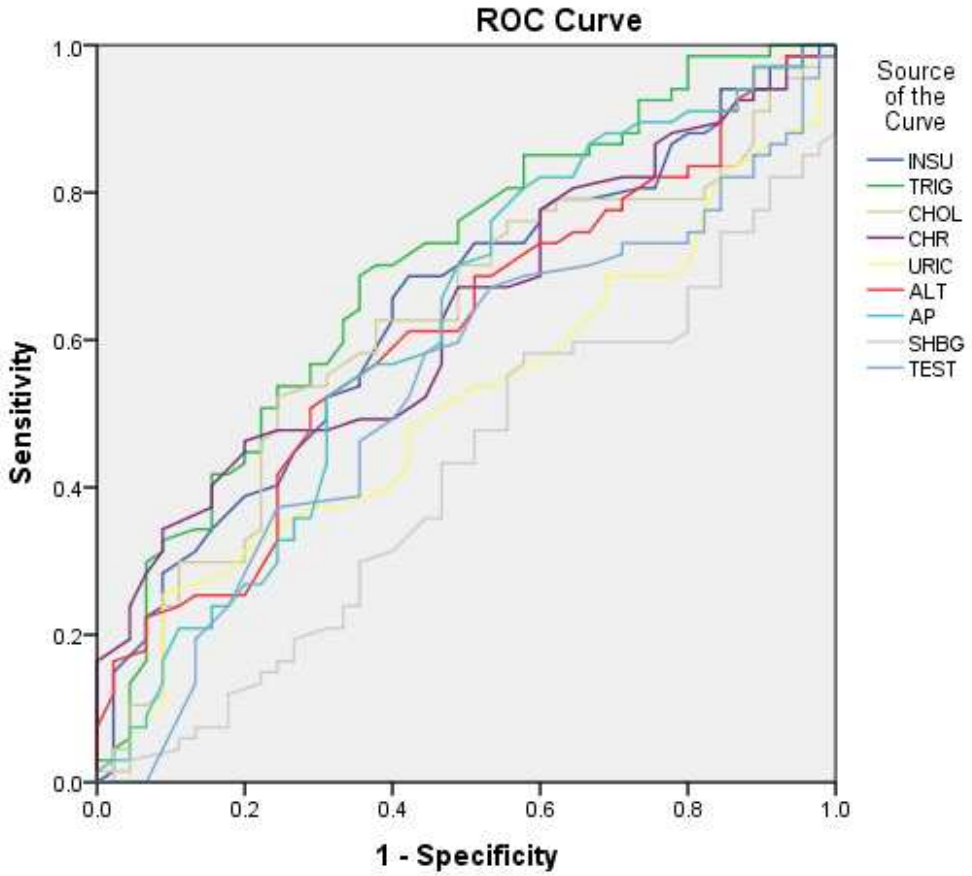
Area Under the Curve-FPG

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.625	.076	.102	.476	.773
TRIG	.646	.075	.056	.499	.793
CHOL	.654	.078	.044	.501	.807
CHR	.560	.070	.433	.422	.698
URIC	.453	.069	.536	.317	.589
ALT	.501	.071	.987	.361	.641
AP	.564	.088	.404	.391	.736
SHBG	.448	.078	.498	.295	.601
TEST	.416	.064	.268	.289	.542



Area Under the Curve-HbA1c (IEC)

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.556	.063	.392	.432	.681
TRIG	.704	.063	.002	.580	.828
CHOL	.617	.064	.075	.491	.744
CHR	.582	.060	.215	.464	.699
URIC	.560	.059	.366	.444	.675
ALT	.639	.055	.034	.532	.747
AP	.675	.066	.008	.546	.805
SHBG	.316	.060	.005	.199	.434
TEST	.540	.063	.546	.417	.662

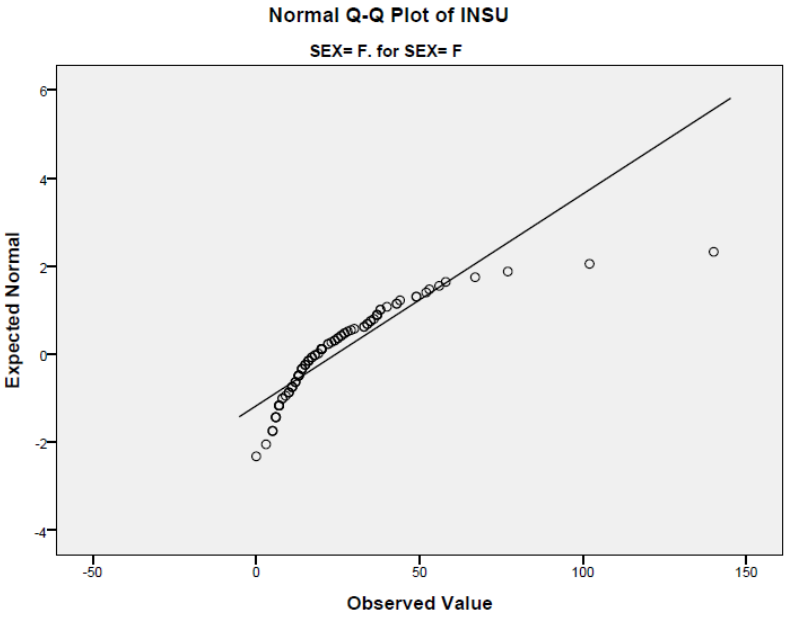


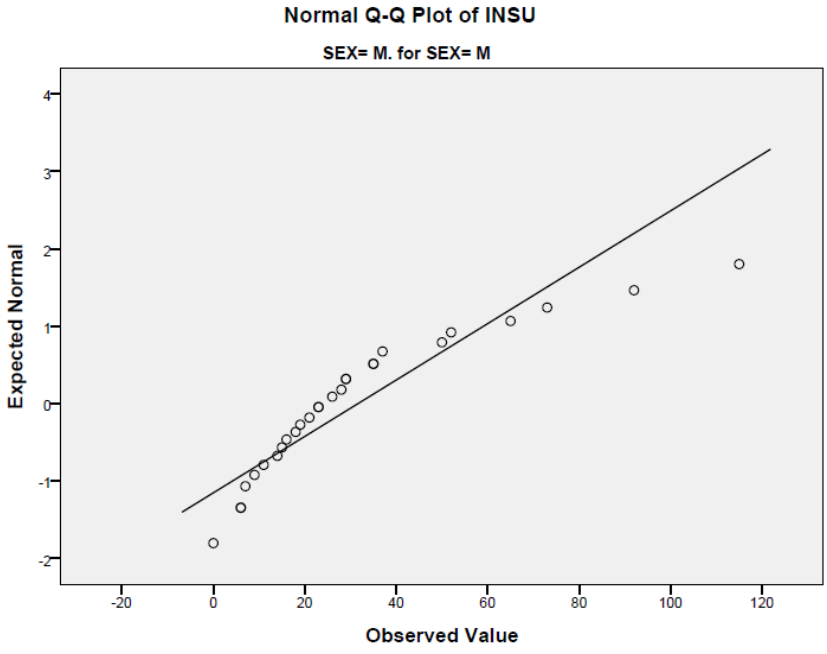
Diagonal segments are produced by ties.

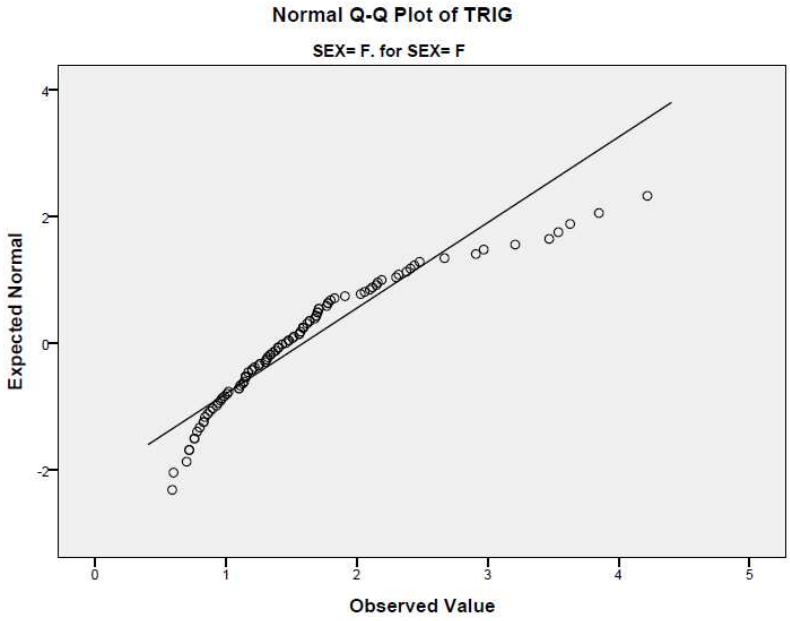
Area Under the Curve-HbA1c (ADA)

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.641	.053	.012	.538	.744
TRIG	.698	.051	.000	.598	.797
CHOL	.617	.054	.036	.512	.723
CHR	.636	.052	.015	.535	.738
URIC	.512	.055	.829	.405	.620
ALT	.609	.054	.051	.504	.714
AP	.618	.055	.035	.509	.726
SHBG	.415	.054	.129	.309	.522
TEST	.536	.056	.523	.426	.645

APPENDIX 4: Q-Q PLOT FOR NORMALITY






Normal Q-Q Plot of TRIG
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Expected Normal
Observed Value
4
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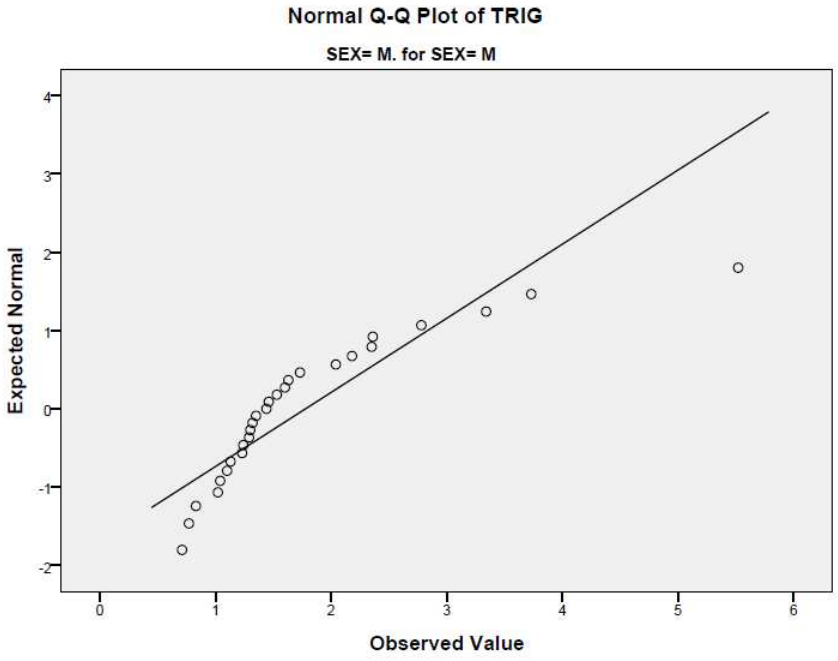
0
-2
0
1
2
3
4
5


Normal Q-Q Plot of TRIG
SEX= M. for SEX= M
Expected Normal
Observed Value
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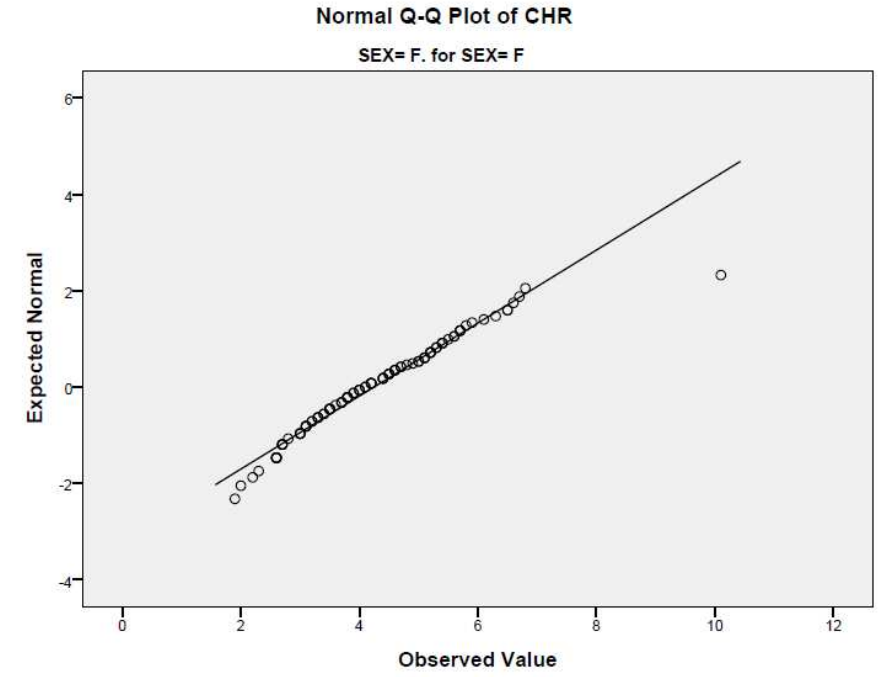
3
2
1
0
-1
-2
0
1
2
3
4
5
6


Normal Q-Q Plot of CHR
SEX= F. for SEX= F
Expected Normal
Observed Value
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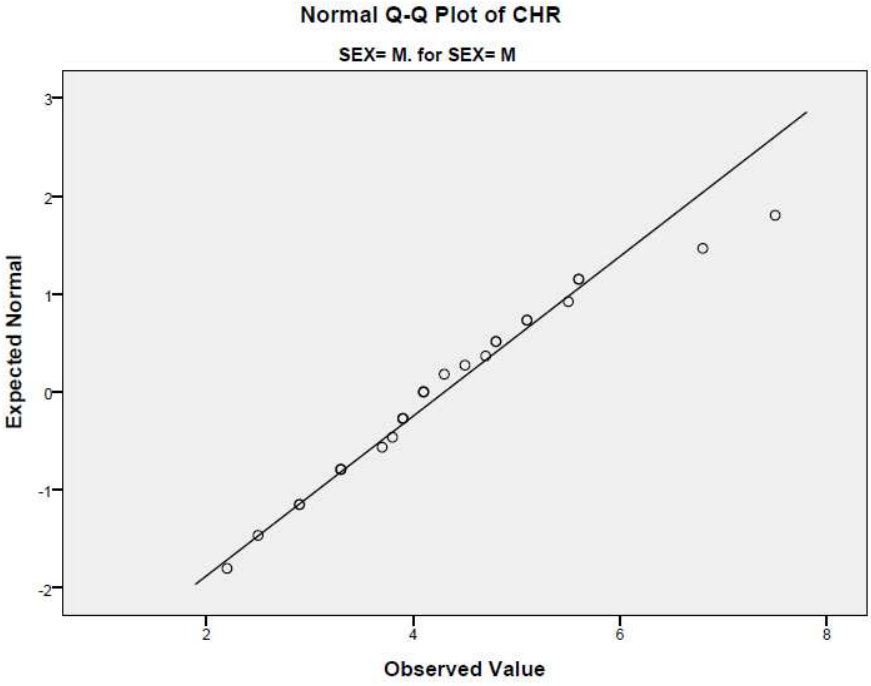
4
2
0
-2
-4
0
2
4
6
8
10
12


Normal Q-Q Plot of CHR
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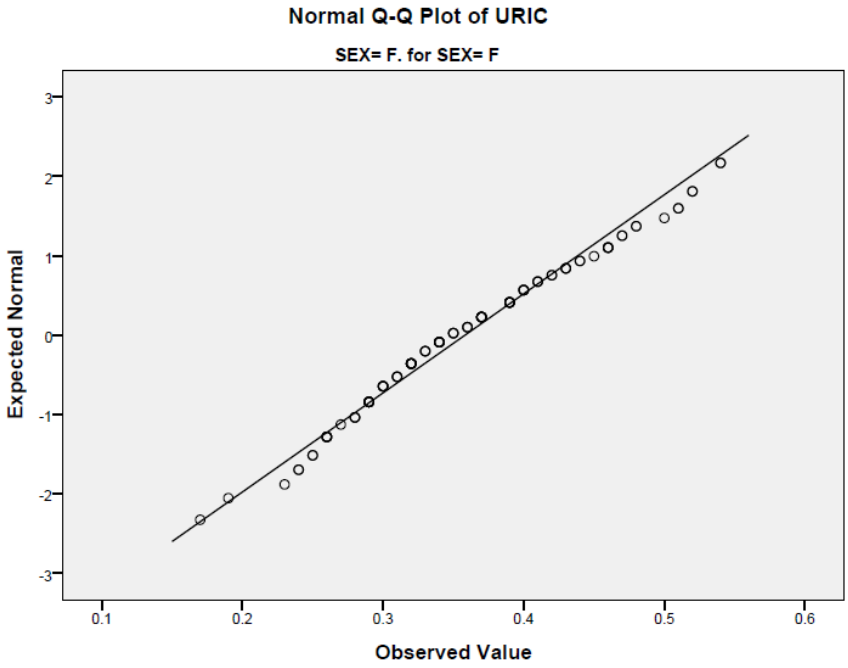
Expected Normal
Observed Value
3
2
1
0
-1
-2
2
4
6
8


Normal Q-Q Plot of URIC
SEX= F. for SEX= F
Expected Normal
Observed Value
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-1
-2
-3
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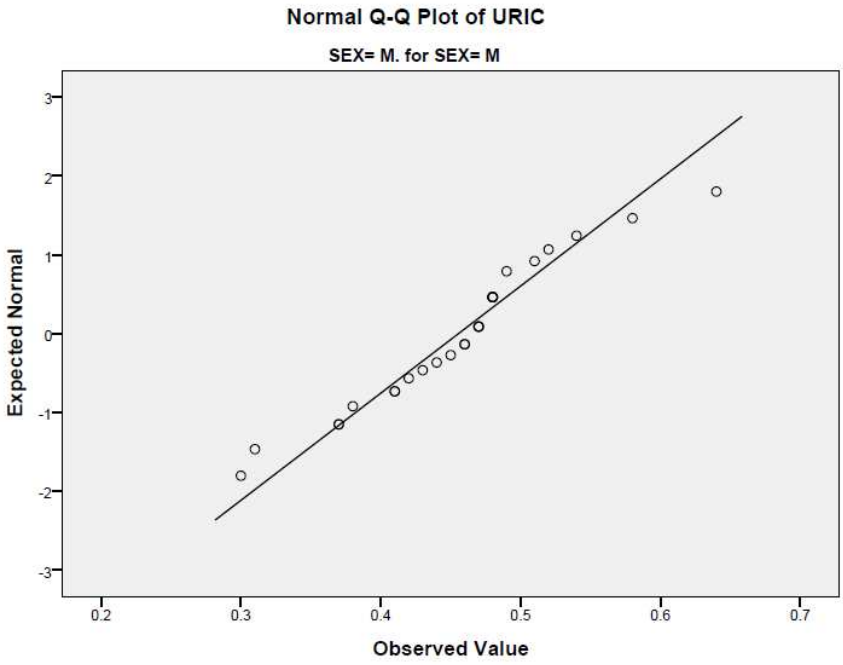
0.2
0.3
0.4
0.5
0.6


Normal Q-Q Plot of URIC
SEX= M. for SEX= M
Expected Normal
Observed Value
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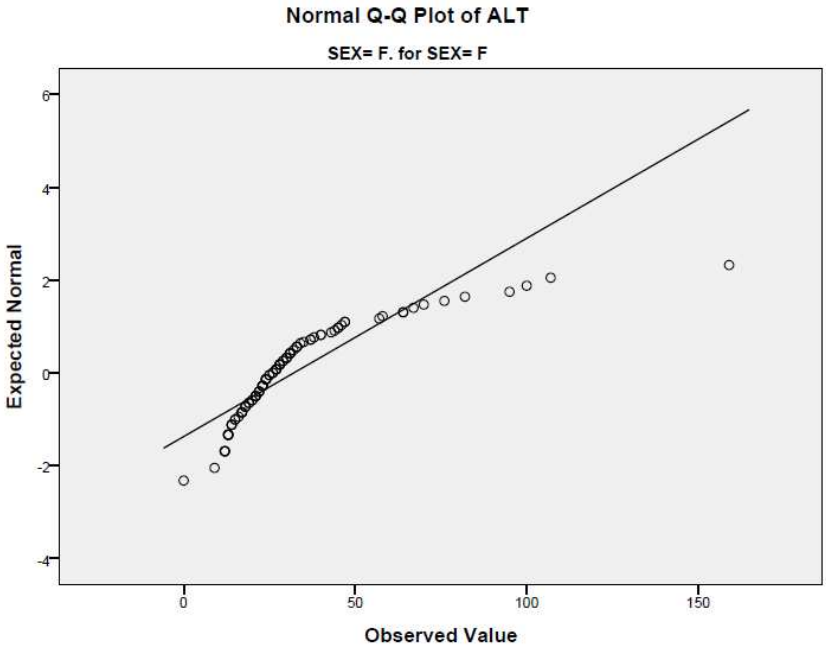
0
-1
-2
-3
0.2
0.3
0.4
0.5
0.6
0.7


Normal Q-Q Plot of ALT
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Expected Normal
Observed Value
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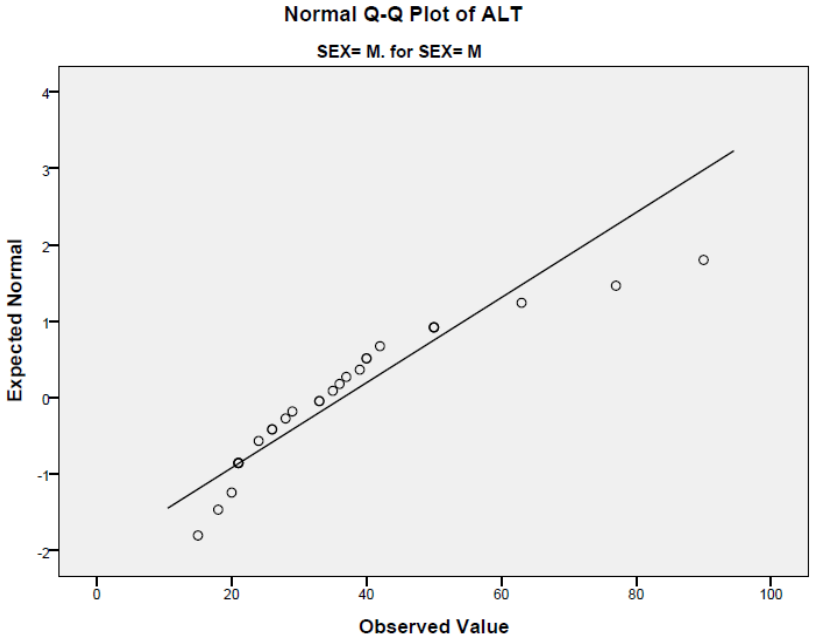
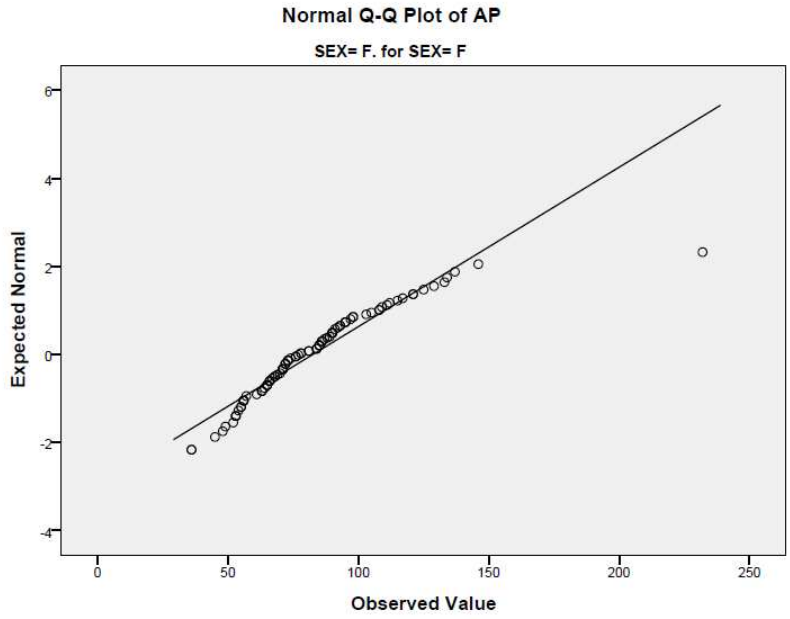
4
2
0
-2
-4
0
50
100
150


Normal Q-Q Plot of ALT
SEX= M. for SEX= M
Expected Normal
Observed Value
4
3
2
1
0
-1
-2
0
20
40
60
80
100


Normal Q-Q Plot of AP
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Expected Normal
Observed Value
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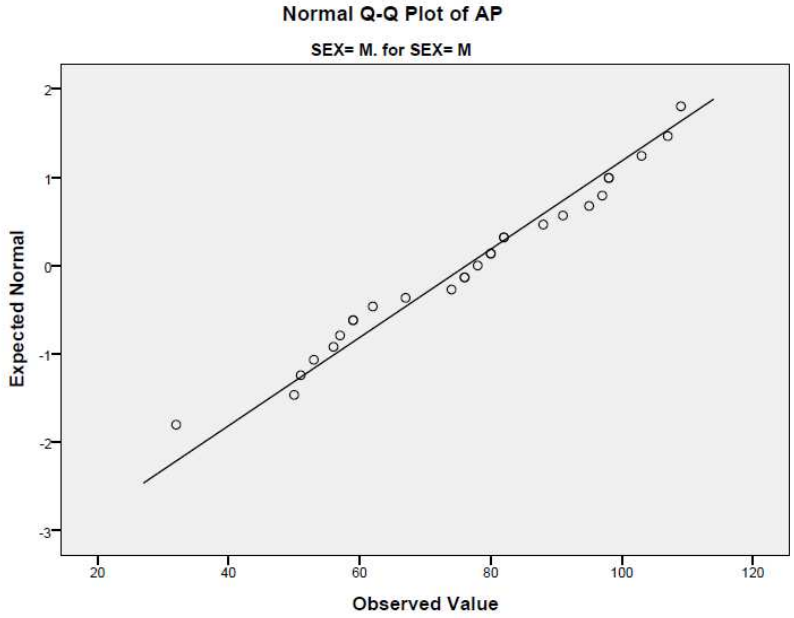
2
0
-2
-4
0
50
100
150
200
250


Normal Q-Q Plot of AP
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Expected Normal
Observed Value
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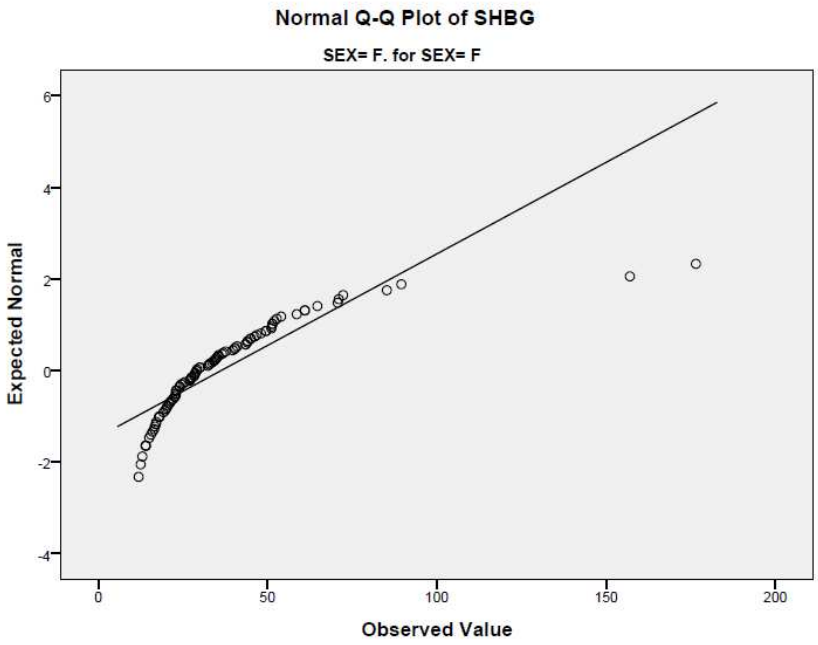
-1
-2
-3
20
40
60
80
100
120


Normal Q-Q Plot of SHBG
SEX= F. for SEX= F
Expected Normal
Observed Value
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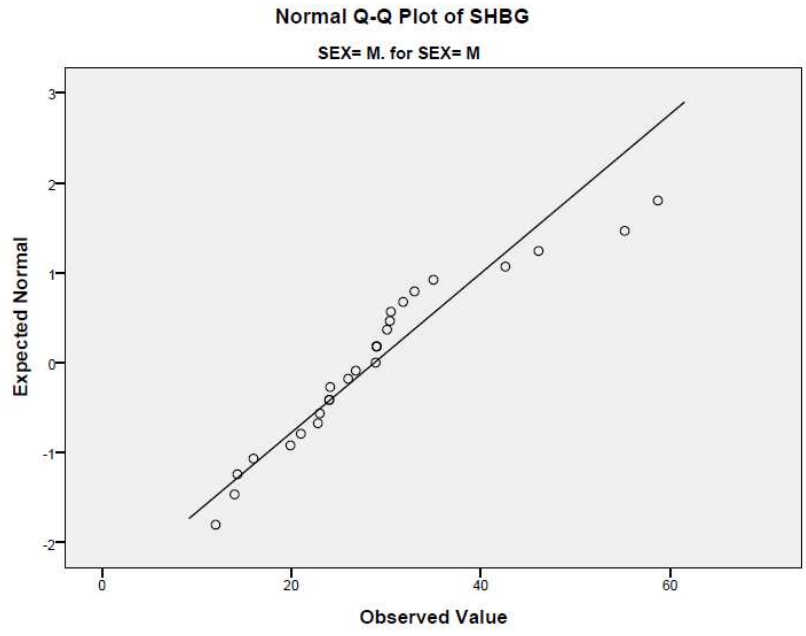
4
2
0
-2
-4
0
50
100
150
200


Normal Q-Q Plot of SHBG
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Expected Normal
Observed Value
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1
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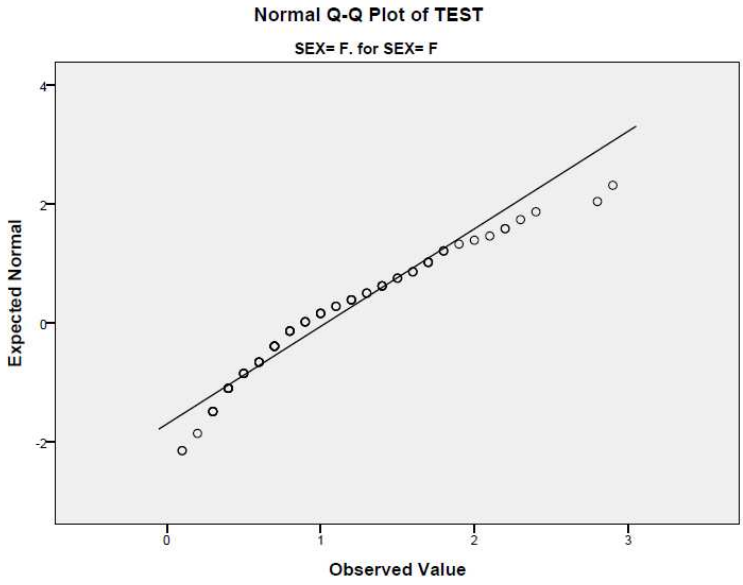
-1
-2
0
20
40
60


Normal Q-Q Plot of TEST
SEX= F. for SEX= F
Expected Normal
Observed Value
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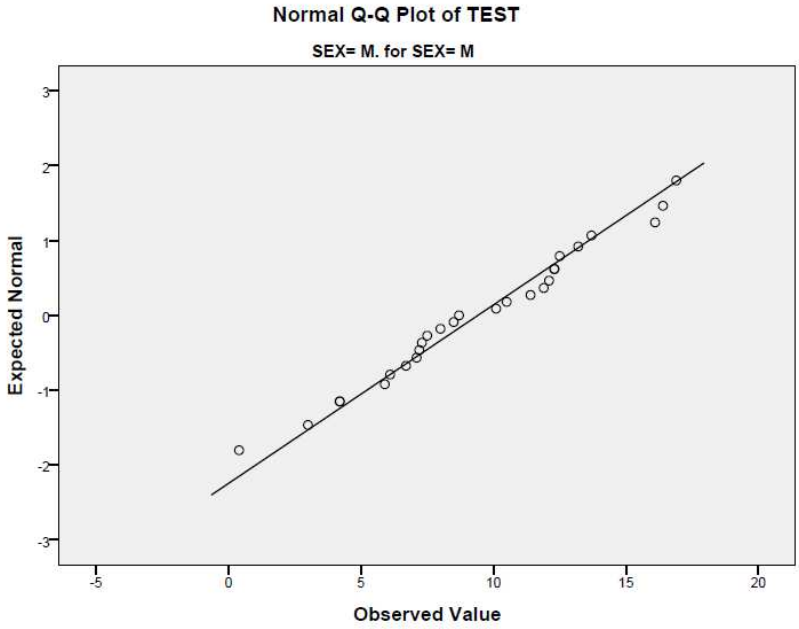
2
0
-2
0
1
2
3


Normal Q-Q Plot of TEST
SEX= M. for SEX= M
Expected Normal
Observed Value
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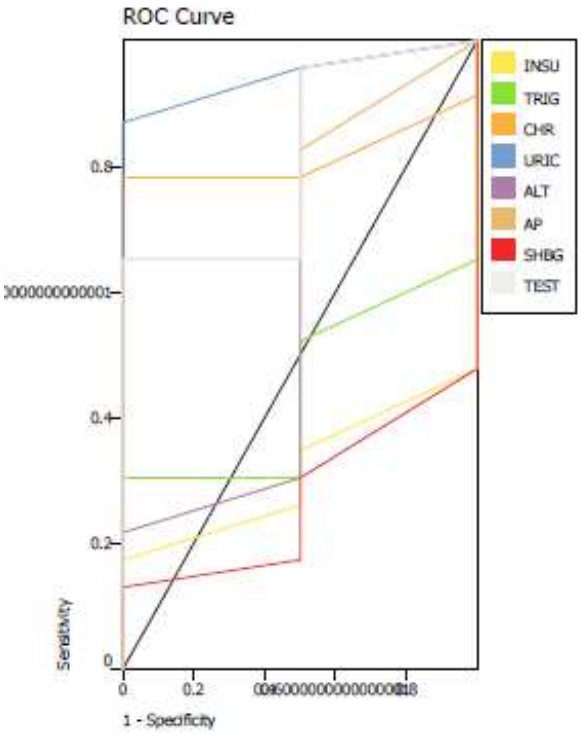
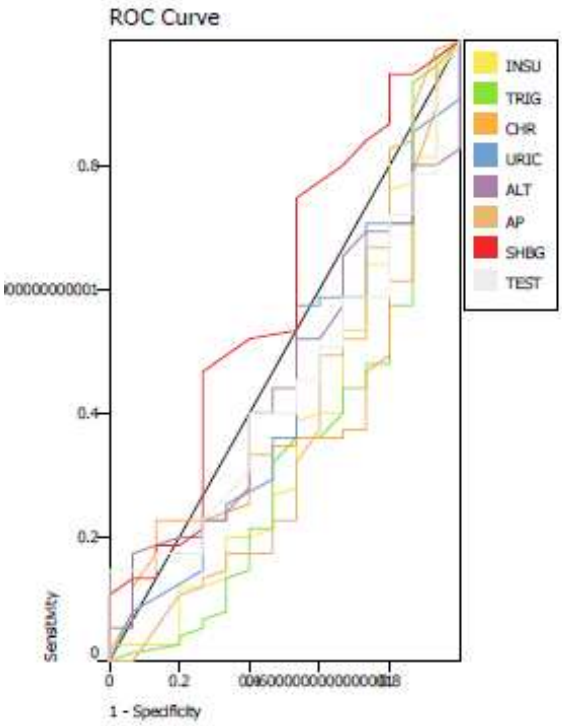
2
1
0
-1
-2
-3
-5
0
5
10
15
20

APPENDIX 5: ROC (Female and Male)





Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.32	.11	.395	.14	.49
TRIG	.43	.15	.764	.22	.65
CHR	.79	.08	.176	.66	.93
URIC	.95	.05	.040	.87	1.02
ALT	.62	.25	.582	.21	1.03
AP	.78	.12	.193	.59	.98
SHBG	.30	.15	.367	.06	.55
TEST	.83	.14	.133	.60	1.05

ROC

```
ROC INSU TRIG CHR URIC ALT AP SHBG TEST BY HbAsc_IEC_Group(0)
    /PLOT CURVE(REFERENCE)
    /PRINT SE.
```

Case Summary

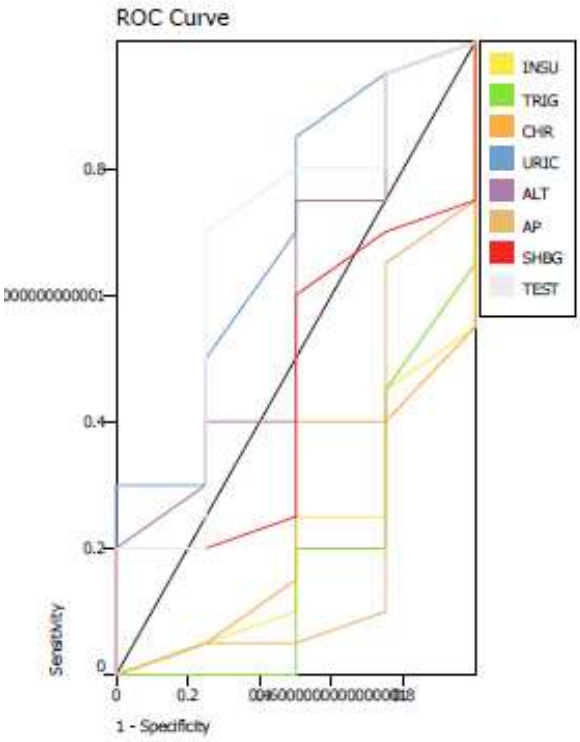
HbAsc_IEC_Group	Valid N (listwise)	
	Unweighted	Weighted
Positive	67	67.00
Negative	21	21.00

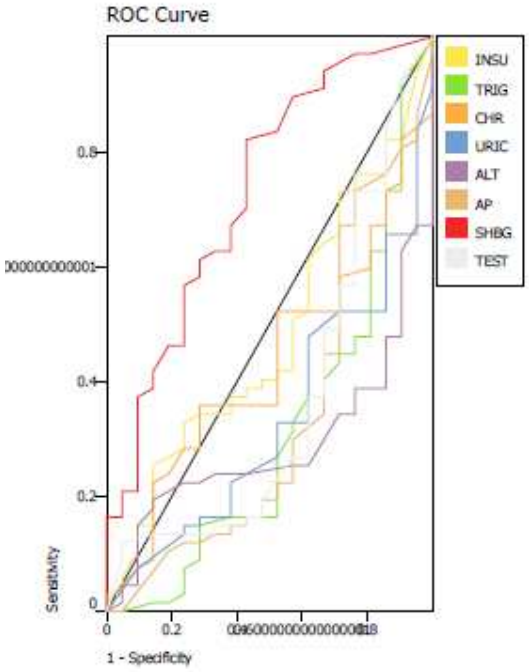
Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.38	.08	.141	.24	.52
TRIG	.34	.08	.045	.20	.47
CHR	.40	.08	.213	.27	.52
URIC	.45	.08	.548	.32	.58
ALT	.46	.08	.611	.33	.58
AP	.39	.09	.187	.24	.54
SHBG	.59	.08	.294	.45	.72
TEST	.47	.08	.685	.34	.59

Case Summary

GLP_group	Valid N (listwise)	
	Unweighted	Weighted
Positive	98	98.00
Negative	17	17.00





Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.23	.12	.096	.03	.43
TRIG	.21	.14	.075	-.02	.45
CHR	.26	.12	.141	.06	.46
URIC	.74	.13	.141	.52	.95
ALT	.59	.17	.588	.31	.86
AP	.23	.16	.088	-.04	.49
SHBG	.44	.14	.699	.21	.66
TEST	.69	.16	.245	.43	.95

ROC

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ROC INSU TRIG CHR URIC ALT AP SHBG TEST BY HbA1b_ADA_Group (0)
    /PLOT CURVE (REFERENCE)
    /PRINT SE.
```

Case Summary

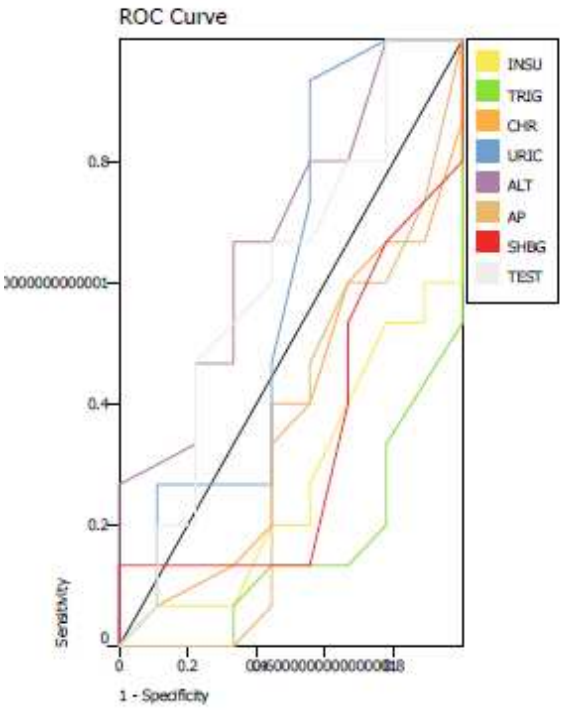
HbA1b_ADA_Group	Valid N (listwise)	
	Unweighted	Weighted
Positive	52	52.00
Negative	36	36.00

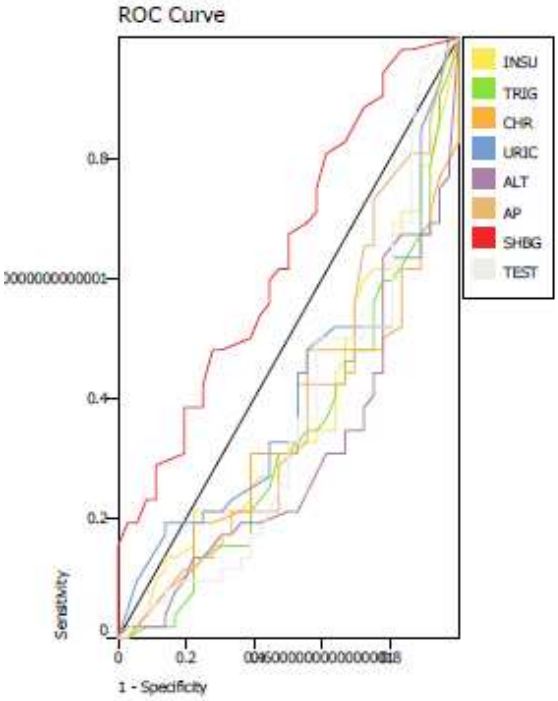
Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.49	.07	.876	.37	.60
TRIG	.31	.07	.010	.20	.43
CHR	.44	.07	.428	.33	.55
URIC	.35	.06	.038	.24	.45
ALT	.30	.06	.006	.21	.39
AP	.35	.07	.038	.23	.47
SHBG	.73	.06	.001	.63	.84
TEST	.39	.07	.120	.26	.51

Case Summary

HbA1c_IEC_Group	Valid N (listwise)	
	Unweighted	Weighted
Positive	87	87.00
Negative	28	28.00





Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.27	.10	.069	.10	.45
TRIG	.16	.08	.006	.02	.29
CHR	.37	.12	.297	.18	.56
URIC	.63	.13	.283	.42	.85
ALT	.70	.11	.114	.51	.88
AP	.34	.12	.200	.14	.55
SHBG	.33	.12	.180	.14	.52
TEST	.63	.12	.297	.43	.83

Area Under the Curve

Variable under test	Area	Std. Error	Asymptotic Sig.	Asymp. 95% Confidence Interval	
				Lower Bound	Upper Bound
INSU	.39	.06	.072	.29	.49
TRIG	.34	.06	.013	.25	.44
CHR	.36	.06	.027	.26	.46
URIC	.42	.06	.217	.32	.52
ALT	.31	.06	.003	.22	.41
AP	.39	.06	.090	.29	.50
SHBG	.64	.06	.027	.54	.74
TEST	.39	.06	.079	.29	.49

Case Summary

HbA1b_ADA_Group	Valid N (listwise)	
	Unweighted	Weighted
Positive	67	67.00
Negative	45	45.00